UNIVERSITY OF ALBERTA

THE EFFECT OF UPSTREAM TREATMENT PROCESSES ON UV INACTIVATION OF MICROORGANISMS IN FILTERED DRINKING WATER

by

RUKEVWE DENNIS UVBIAMA ©

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DEDICATION

To my dad, Daniel Uvbiama, for the sacrifices he made for me to have this degree and to my dearest family and friends for their unending love and prayers.

ABSTRACT

UV radiation is now an acceptable disinfectant for drinking water. Particle-associated microorganisms reduce the effectiveness of UV disinfection. The main objective of this study was to determine and quantify the effect of aggregation on the UV inactivation of microorganisms in filtered and backwash water. This bench-scale study used river water containing *Bacillus subtilis* spores, which was coagulated and settled and then filtered through a sand-anthracite coal filter until break through occurred. Homogenization was used to break up the aggregates in order to observe the effect of aggregation. The results of this study suggest that under sub-optimal conditions, a fraction of the spores that broke through a laboratory-scale filter column were aggregated, and thus, the UV inactivation rate constant was underestimated. A significant difference was found between the inactivation of the spores added directly to raw water relative to the inactivation of the spores added to filtered water containing particles.

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ABBREVIATIONS

ATCC	American Type Culture Collection
AWWA	American Water Works Association
CFU	Colony Forming Units
HAA	Haloacetic Acids
ID	Internal Diameter
IESWTR	Interim Enhanced Surface Water Treatment Rule
LT2ESWTR	Long Term 2 Enhanced Surface Water Treatment Rule
Log	Logarithm
LSD	Least Square Difference
Magn.	Magnification
N/A	Not Applicable
ND	Not Done
NS	Not Significant
NTU	Nephelometric Turbidity Unit
S	Significant
SWTR	Surface Water Treatment Rule
THMs	Trihalomethanes
PCTE	Polycarbonate Track Etched
PVC	Polyvinyl Chloride
US	United States
USEPA	United States Environmental Protection Agency
UV	Ultraviolet

SYMBOLS

a	Absorption coefficient
c	Speed of light
C	Concentration of disinfectant
C_p	Center point of irradiated sample
d	Sample depth
D	UV dose
D_s	UV dose at shoulder of inactivation curve
D_{lag}	Lag UV dose
d_p	Particle diameter
E_λ^t	Transmitted light
E_λ^o	Incident light
h	Planck's constant
hv	Photons
I	Irradiance
I_{avg}	Depth Averaged UV irradiance
k_m	Rate constant, multi-target
k_s	Rate constant, series event
k_{LSD}	Least Square Difference factor k
l	Path length
L_d	Distance between lamp surface and top of liquid samples
$LSD_{ij}(S)$	Scheffe's Least Square Difference
MS_E	Mean square error
n	Number of sample points
n_c	Number of targets
n_d	Dilution coefficient
n_i, n_j	Number of factor B
$n_p(d_p)$	Number of particles
N	Number of survivors
N_f	Final number of viable microorganisms

N_i	Initial number of viable microorganisms
N_p	Total number of particles
p-value	Probability of type I error
Q	Energy of radiation
t	Time
T_λ	Transmittance at specific wavelength
t_s	Treatment levels
W	Watts
x, y, z	Orthogonal directions
$\bar{y}$	Average

GREEK SYMBOL

λ	Wavelength

1 INTRODUCTION

1.1 BACKGROUND

The USEPA's proposed Long Term II Enhanced Surface Water Treatment Rule (LT2ESWTR) has identified ultraviolet (UV) irradiation as an acceptable technology for achieving additional *Cryptosporidium parvum* inactivation credit by drinking water treatment systems (USEPA 2003a). This regulation allows treatment systems to have alternative and/or a combination of treatment technologies including chlorine, ozone, and membrane filtration for microbial reduction and disinfection by-products (DBP) control (USEPA 2003a). This change was necessitated because the chlorination of drinking water containing natural organic matter (NOM) could lead to the formation of DBP such as haloacetic acids (HAA), trihalomethanes (THM), and keto acids (Dalvi et al. 2000; Krasner et al. 1989; Rook 1977), which are known to have deleterious health effects (Marx 1974). In addition, the formation of bromate, also a probable carcinogen, by ozone in the presence of NOM and other precursors in source water has reduced the incentive to install new ozonation systems (Glaze 1987). What has created even more concern about carcinogens in water is the possible long-term effect of low concentrations of DBP on humans drinking chlorinated and ozonated water. Moreover, chlorine, which is a strong oxidant, has been found to be effective in inactivating *Giardia lamblia* but is ineffective against *C. parvum* even though long contact times were needed for the former (Fayer 1995; Korich et al. 1990). Consequently, UV disinfection became an attractive disinfectant method when low UV doses were shown to inactivate *C. parvum*, *G. lamblia* (Bukhari et al. 1999; Clancy et al. 2000; Craik et al. 2001; Korich et al. 1990) and some viruses without leaving DBP after inactivating the pathogens.

According to the LT2ESWTR, applied UV doses of 11 and 12 mJ/cm^2 are sufficient to achieve 3 log reduction credit for *G. lamblia* and *C. parvum*, respectively, when applied post-filtration (USEPA 2003a). This criterion is based on the outcomes of several studies demonstrating that exposure to UV reduced the infectivity of *C. parvum* oocysts at relatively low doses (Bukhari et al. 1999; Clancy et al. 2000; Craik et al. 2001). In these studies, samples of relatively clean water were seeded with purified parasites preparations (i.e. parasite preparations were added to the water) and exposed to predetermined UV doses by using a collimated beam apparatus. The parasites in these bench-scale studies were assumed to be well-dispersed in the experimental water matrix. Hence, the potential for interaction with particulate matter may not be accounted for in these specified doses if particular matter is present in the water. However, the LT2ESWTR criterion applies mostly to properly-filtered surface water low in particulate matter as well as unfiltered water that meets the filtration avoidance criteria (40 CFR 141.71) (USEPA 1989).

In conventional water treatment, coagulation and flocculation processes promote the aggregation of particulate matter and microorganisms in the source water so that these are readily removed by settling and granular media filtration (AWWA 1999). Many waterborne disease outbreaks have been associated with periods of sub-optimal operation of coagulation, flocculation, settling, and filtration processes and turbidity excursions in drinking water treatment systems (Hrudey and Hrudey 2004; MacKenzie et al. 1994). The outbreak of cryptosporidiosis in North Battleford, Saskatchewan, Canada is a typical example of how sub-optimal operation of treatment systems led to an outbreak. In this case, the failure of the up-flow clarifier (solid contact unit) to form a floc blanket because of low turbidities in the raw water led to the improper removal of turbidity and resulted in the break through of *C. parvum* in the filtered water (Hrudey and Hrudey 2004). About 5,800 to 7,100 people were estimated to have been infected with gastrointestinal cryptosporidiosis (Hrudey and Hrudey 2004). In these instances, some fraction of the microorganisms that broke through the filtration barrier may have existed as aggregates, bound to other microorganisms (microbe-microbe association), particulate matter (microbe-particle association) or as mono-dispersed microorganisms. This possible scenario needs to be investigated because the specified UV doses in the LT2ESWTR and current design practice of UV reactors do not recognize the possibility of aggregation in break through water.

Seeding (microorganism addition) is a necessary experimental artifact in UV disinfection studies because the concentrations of microorganisms of interest in filtered or unfiltered source water are usually too low to permit measurement of inactivation. Most studies on microbe-particle and microbe-microbe association have been conducted with wastewater (Emerick et al. 2000; Emerick et al. 1999; Jolis et al. 2001; Liltved and Cripps 1999; Loge et al. 2001; Loge et al. 1996; Loge et al. 1999; Qualls et al. 1983; Qualls and Johnson 1985), unfiltered (Passantino et al. 2004) and filtered (Batch et al. 2004; Craik et al. 2001) drinking water. In the drinking water studies, the sample water matrices were seeded with prepared microbial preparations. Outbreaks have been associated with periods of sub-optimal operation of treatment systems during which the public was exposed to pathogenic microorganisms that broke through the treatment system. The state of dispersion of the break through microorganisms is currently not known. Consequently, the effectiveness of UV disinfection may be reduced given that UV is relatively ineffective against shielded or particle-associated microorganisms. Research into the state of dispersion of microorganisms that break through a sub-optimal multiple barrier treatment process and into the impact of the upstream processes is on the inactivation rate of test microorganisms is needed. Studies that have utilized seeding of microorganisms and particle into different water matrices found no significant impact of particles on UV inactivation (Amoah et al. 2005; Batch et al. 2004; Passantino et al. 2004). To accurately account for the impact of particles on UV disinfection, the effects of aggregation of microorganisms and particulate matter in natural water, during coagulation, flocculation and settling, need to be considered when designing UV reactors or specifying dose requirements.

1.2 HYPOTHESIS

The hypothesis for this research was that some fraction of the microorganisms in filtered water that break through a sand-media filter will be aggregated. Therefore, aggregation and the presence of particulate matter will affect the UV inactivation of microorganisms in filtered water. In addition, adding microorganisms directly into filtered water containing particulate matter may not be representative of aggregation that drinking water UV disinfection studies attempt to investigate.

1.3 RESEARCH OBJECTIVES

1.3.1 Objectives

The specific objectives of this study were to

- Determine whether microorganisms that break through sand-media filters are aggregated and the extent of the aggregation,
- Determine and quantify the effect of aggregation on the UV inactivation of microorganisms in filtered and backwash water,
- Determine whether adding microorganisms directly to raw river water may be a better representation of aggregation,
- Use membrane filters and particle-size distribution to directly measure the aggregation of microorganisms in filtered water, and
- Examine microscopic images of microorganisms on membrane filters to visually observe possible effects of aggregation.

1.3.2 Tasks

The following tasks were done to achieve the above objectives

- Design, build and operate a laboratory scale constant-head dual media filter to investigate the possible effect of upstream water treatment processes, like coagulation, flocculation, and settling, on UV inactivation.
- Use a model microorganism, *B. subtilis* spores, to investigate aggregation of microorganisms in filtered and backwash water under sub-optimal filtration conditions. Backwash water was also investigated because backwash will contain more aggregated microorganisms than filtered water, and hence, the effects of aggregation will be more visible in the backwash water.
- Examine microbial and particle-size distribution of filtered water and determine the size of particles that could impact the UV inactivation of microorganisms.
- Compare non-linear models of UV irradiated *B. subtilis* spores in filtered water by using statistical and non-statistical techniques.
- Use scanning electron microscopy to visually examine the aggregation of *B. subtilis* on polycarbonate track etched membrane filters.

1.4 OUTLINE OF THESIS

This thesis is organized into five chapters. Chapter 1 provides a general background and describes the research problem and study objectives. A review of relevant literature is presented in Chapter 2. Chapter 3 provides detailed information about the materials, experimental apparatus, procedures, and techniques used to accomplish the objectives of this thesis. Experimental results are presented in Chapter 4 and discussed in Chapter 5. The conclusions arising from the results and discussions of Chapter 5 are presented in Chapter 6. The appendixes contain raw data, sample calculations, results of supporting experiments, and SEM images of membrane filters.

2 LITERATURE REVIEW

This chapter reviews pertinent literature on UV radiation, mechanisms of UV inactivation, UV radiation sources, UV disinfection of drinking water, UV inactivation of *B. subtilis* spores, UV inactivation kinetic modeling, and the effects of particulate matter on UV inactivation of microorganisms. At the end of this chapter, the need for this research is discussed.

2.1 DRINKING WATER REGULATIONS

Drinking water regulations in the United States are the most developed in North America, hence, the USEPA regulations will be reviewed in this section. A review of the past and current drinking water regulations is necessary to explain the issues that have driven the industry to implement UV disinfection. In addition, an introduction to the regulations will help explain some of the recommendations of this thesis.

Concerns about higher levels of cancer among people who had used filtered and chlorinated water from the Mississippi informed the need for the federal regulation of drinking water in the United States (Okun 1996). The 1974 Safe Drinking Water Act (SDWA) was the first drinking water regulation that was enforceable at the federal, state and local jurisdictions in the US (SDWA 1974). The SDWA has had several amendments, but the two major amendments were in 1986 and 1996. Although the 1986 amendment included most of the initial regulations from the 1974 SDWA, the major focus of the 1986 amendment was to reinforce the USEPA in the regulating process and provide for groundwater protection. The Milwaukee incident of 1993 in which more than 400 000 people were infected by *C. parvum* (MacKenzie et al. 1994) spurred another amendment to the SDWA in 1996 (AWWA 1999).

The USEPA has put forward several regulations in accordance with the SDWA and its amendments. The 1989 Surface Water Treatment Rule (SWTR) established treatment requirements for *G. lamblia*, bacteria and viruses. The rule provided concentration × contact time (Ct) tables for free chlorine, monochloramine, chlorine dioxide and ozone (USEPA 1989). The 1998 Interim Enhanced Surface Water Treatment Rule (IESWTR) was proposed after the 1996 amendments to the SDWA. Under this rule, public water systems using surface water or groundwater under the direct influence of surface water were required to achieve > 99% removal of *C. parvum* based on turbidity reduction. Concerns about the carcinogenic effects of DBP formed the basis for the 1998 Stage 1 Disinfection By-products Rule (Stage 1 D/DBPR) and the 2003 Stage II Disinfection By-products Rule. This regulation sets limits for the maximum residual disinfectant for free and combined chlorine and chlorine dioxide; and maximum contaminant level goals for THMs, HAA, chlorite, and bromate.

In addition to improving protection against the microbial protozoan *C. parvum*, the Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR) requires measures to reduce the risk of DBP. This rule, which uses the Interim Enhanced Surface Water Treatment Rule (IESWTR) as its foundation, applies mostly to treatment systems serving populations of 10 000 people or less.

With the introduction of the 2003 LT2ESWTR, more stringent regulations for the removal of *C. parvum* based on a bin system were established. For the first time, the USEPA created a set of guidelines for microbial reduction that includes primary disinfection using chlorine dioxide, ozone, or UV radiation. A guidance manual was also developed for each primary disinfectant. A draft of the proposed UV disinfection guidance manual (UVDGM) was published in June 2003. The UVDGM not only gathers the past and current technical knowledge on UV inactivation of microorganisms but also attempts to standardize the planning, design, operation, and validation of UV installations for drinking water purposes. The manual is organized into five major sections. A summary of the important aspects of relevant drinking water regulations is provided in Chapter One. The second chapter gives an overview of UV disinfection, including information on UV radiation, the response of microorganisms to UV radiation, UV reactors, the effects of water quality on UV disinfection, and the formation of by-products. Chapter Three deals with subjects pertaining to the planning and design of UV installations. Chapter Four discusses the validation testing of UV reactors. Finally, Chapter Five describes the start-up and operation of UV reactors with specific recommendations for off-specification, monitoring and reporting (USEPA 2003b).

A dose table (*I*t*) which specifies the UV dose required to inactivate *C. parvum*, *G. lamblia*, and viruses is provided as recommended by the Federal Advisory Committee (FACA) (USEPA 2003a; USEPA 2003b). Although the final draft of the UV disinfection guidance manual is scheduled to be released in December 2005, several issues remain, including many others, particle effects, safety factors, and an appropriate surrogate for validation of UV reactors for viruses and *C. parvum*.

2.2 UV RADIATION

2.2.1 Electromagnetic spectrum

The electromagnetic spectrum is made up of radio waves, microwaves, infrared light, visible light, ultraviolet light, x-ray and gamma rays. Visible light was first shown by Heinrich Hertz to have the same speed as radio waves, as theorized by James Clark Maxwell in his electromagnetic theory, proving that light has wave-like properties. In 1900, Max Planck quantified the energy of radiation by assuming that energy would be proportional to the frequency if the radiation were in discrete "chunks" or "packets". This relationship can be expressed as

$$Q = \frac{hc}{\lambda}, \qquad \text{Equation 2-1}$$

where Q is the energy of the radiation in joules; h is Planck's constant (6.620×10^{-34} Js), c is the speed of light (2.998×10^{8} m/s) and λ, is the wavelength of the radiation in meters (Ryer 1997). Based on the work of Planck, Albert Einstein proposed that energy in radiation is delivered in packets, and therefore, light must be made up of particles, which he called *photons* (Bolton 1999). Therefore, light can be said to have both wave and particle-like properties.

The components of the electromagnetic spectrum can be described by their wavelength, energy and frequency. As shown in Figure 2-1, radio waves have very long wavelengths whereas gamma rays have short wavelengths. Because energy is inversely proportional to wavelength, radio waves have the least energy whereas the gamma rays have the highest energy.

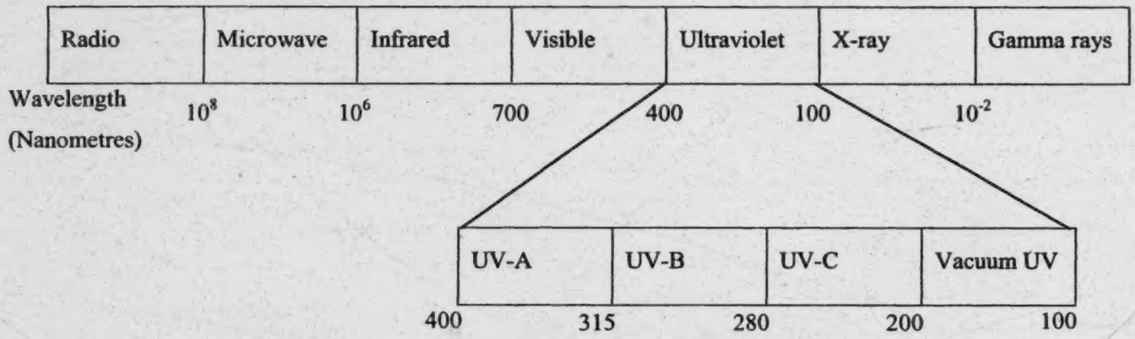

Figure 2-1 The electromagnetic spectrum (adapted from Jagger 1967).

2.2.2 UV radiation Ranges

UV radiation is between the visible and X-ray portion of the electromagnetic spectrum with a wavelength between 400 and 100 nm (Jagger 1967). UV radiation can be classified into four different segments partly based on its effects on humans. UV-A (315 to 400 nm) is commonly used in tanning salons and for making fluorescent materials fluoresce (Dyer 1997). UV-B (280 to 315 nm) can cause sunburns and skin cancer. UV-C (200 to 280 nm) is known for its ability to destroy cells because of its high energy levels. UV radiation with a wavelength of 100 to 200 nm is readily absorbed in air and water; hence, a vacuum is required to propagate UV with a wavelength in this range. Thus, it is referred to as *vacuum ultraviolet light* (VUV).

2.3 MECHANISMS OF UV INACTIVATION

2.3.1 Electronic Excitation

UV disinfection stems from two basic principles: the laws of photochemistry and electronic excitation. According to the first law of photochemistry (Grotthus-Draper Law), a photochemical reaction cannot occur unless a photon of light is absorbed (Smith and Hanawalt 1969). In order for microorganisms to be inactivated, their cells must absorb UV radiation. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), components of most living organisms, absorb UV at wavelengths between 240 to 300 nm, with peak absorption at about 265 nm (Harm 1980). This UV absorbance peak coincides with the UV-C wavelengths which can be reliably produced with commercially available mercury vapor UV lamps. Because this range is more effective in inactivating living cells when compared to UV-A and UV-B, it is usually referred to as *germicidal UV* (Bolton 1999; Harm 1980; Jagger 1967; Smith and Hanawalt 1969) .

When a photon of UV radiation is absorbed by an electron in an atom, the energy in the electron is increased by the same amount of photon energy (Harm 1980). An electron with an increased energy is said to be *excited*. An excited electron can be taken from the ground state to either the first, second or even higher excited state depending on the amount of energy possessed by the absorbed photon. Electrons continue to gain energy until a limit is reached. The energy possessed by the electron at this limit is called the *ionization energy*. The difference between the ionization energy and the energy of the electron at the ground state is called the *ionization potential* (Jagger 1967). Electrons having energies less than the ionization potential fall back to the ground or a lower excited state with the emission of a photon. This emission is usually accompanied by the emission of light, a process commonly described as *fluorescence*. Usually, the emitted photon possesses energy less than that which was absorbed. However, when a photon with energy greater than the ionization potential excites an electron, the electron escapes from the atom. Therefore, the atom is said to be *ionized* (Jagger 1967).

In molecules, however, the absorption of a photon leads to increases in their vibrational, translational and rotational energy. Because the electrons in a molecule are shared, molecular excitation is somewhat different than electronic excitation. In contrast to the single energy band produced by electrons, molecules give a wide band of spectral emission (Jagger 1967).

2.3.2 Absorption and Action Spectrum of DNA and RNA

DNA and RNA are made up of nitrogen-containing heterocyclic aromatic compounds called *pyrimidines and purines*. Both DNA and RNA contain four bases: two pyrimidines and two purines. In DNA, the two pyrimidines are cytosine and thymine whereas RNA has cytosine and uracil. The purines in DNA and RNA are the adenine and guanine bases. Figure 2-2 shows the chemical structure of the bases in DNA and RNA. From this point forth, UV-C light shall be referred to as just *UV radiation* with a wavelength of 200 to 280 nm unless otherwise stated. The chemical bases (Figure 2-2) are known to absorb UV radiation in the 200 to 300 nm range (Harm 1980). The absorption of UV radiation peaks at around 260 nm (Figure 2-3). Therefore, these bases would be the primary target if the living cells are to be inactivated.

2.3.3 Effect of UV radiation on DNA and RNA

Most microorganisms are inactivated by germicidal UV radiation through photochemical reactions of the pyrimidine bases. When the DNA and RNA absorb UV photons, the adjacent pyrimidines bases are covalently linked. The 5, 6 double bonds of the pyrimidine bases become saturated, thereby forming a four-member ring structure. The resulting cycloaddition product is referred to as *cyclobutane dipryrimidine* or *pyrimidine dimer* (Friedberg et al. 1995). A section of the DNA or RNA molecule that has been damaged due to UV exposure is called a *lesion.* A DNA or RNA strand separated by at least one base pair can have multiple lesions. The photoproduct of dimerization exists in 12 possible isomeric forms, but only four of these products have significant yields. These include the *cis-syn*, *cis-anti*, *trans-syn* and *trans-anti*, with the *cis-syn* being the most predominant (Friedberg et al. 1995). Of the two pyrimidine bases in the DNA, thymine is the stronger absorber at 254 nm and forms thymine dimers on absorption of UV photons (Bolton 1999). Thymine dimers are the most significant lesions involved in UV inactivation of microorganisms (Figure 2-4).

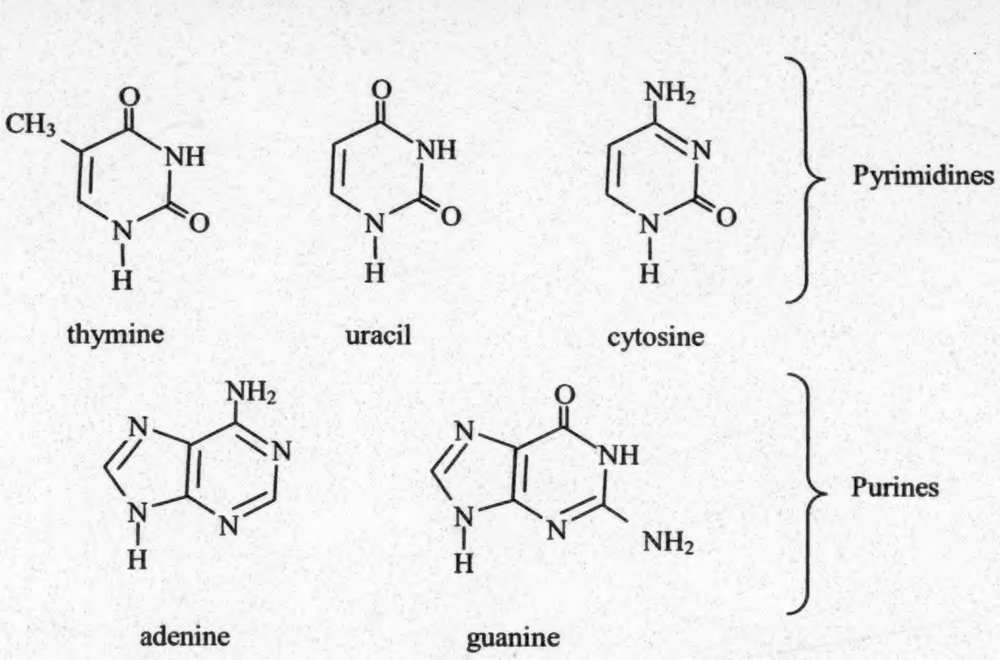

Figure 2-2 Chemical structure of pyrimidines and purines of DNA and RNA bases (Blackburn and Gait 1996)

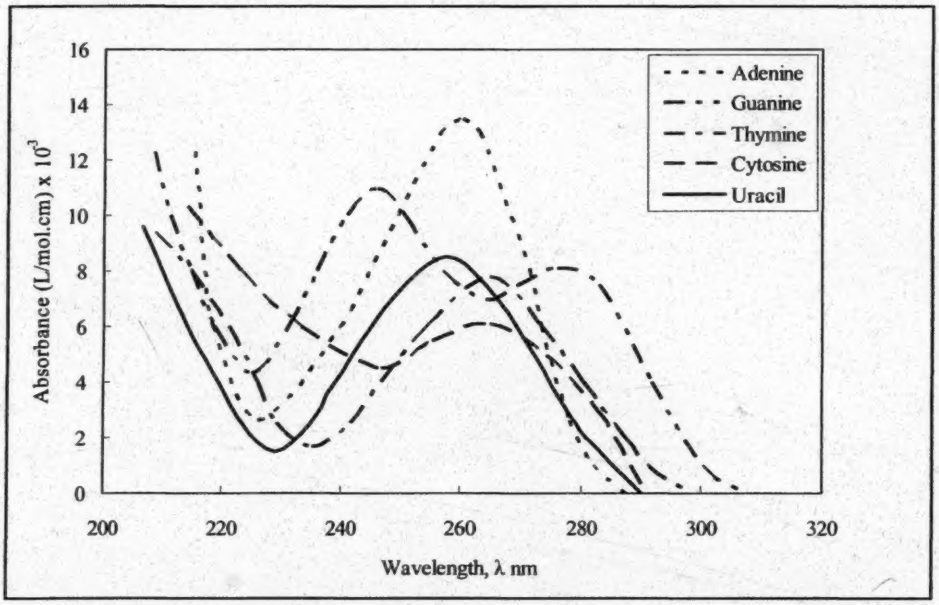

Figure 2-3 Absorption spectra of pyrimidine and purine base in DNA and RNA (adapted from Jagger 1967).

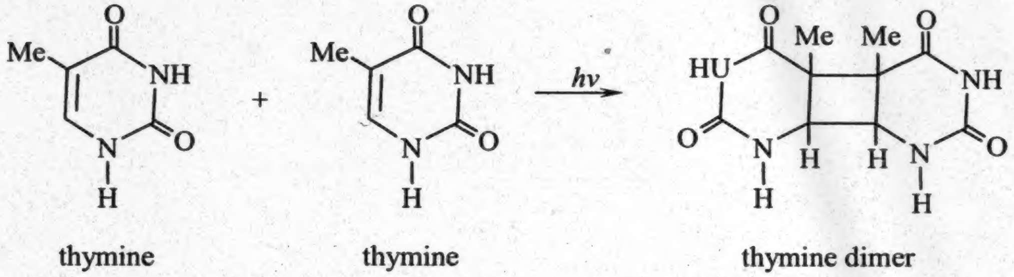

Figure 2-4 Photochemical dimerization of two thymine bases (Bolton 1999).

Lesions prevent the replication of DNA. Watson and Crick (1953) proposed the double helix structure of DNA. In their proposed structure, a purine or pyrimidine is hydrogen-bonded to a complement base in the complementary strand of the double helix. For double-stranded DNA to replicate, the DNA first splits into two separate strands, and then the phosphodiester linkages join the complementary pyrimidine or purine bases to the two separate strands. Thus, two copies of the DNA are formed. The presence of lesions on a DNA strand will prevent replication of the DNA. In microorganisms with the ability to repair their DNA, repair is usually by light or dark reactivation (Friedberg et al. 1995). When a microorganism cannot repair its DNA, the microorganism cannot replicate. A cell that cannot replicate cannot cause disease and, hence, is considered to be *inactive*.

In bacterial spores, the photoproduct is somewhat different than the pyrimidine dimer formed in a DNA. In this case, a non-cyclobutane pyrimidine dimer is formed. One of the major photoproducts of irradiated of *B. subtilis* spores is 5'-thyminyl-5,6-dihydrothymine (Setlow 1992). A major contributor to this difference is the interaction between the DNA and acid-soluble proteins (Driks 1999). Some microorganisms including *B. subtilis* seem to have no ability to repair their DNA (Masschelein 2002).

2.4 UV RADIATION SOURCES

UV lamps are used to generate monochromatic and polychromatic UV radiation. The various types of UV lamps including temperature radiators, gas discharge, electroluminescence, and light emitting diodes are classified according to the way in which the light is produced. Because most UV lamps used in water treatment use gas discharge, only these types of lamps will be discussed.

In gas discharge lamps, light is generated when an excited electron of a filler gas falls from a higher excited state to either a lower excited or ground state as described earlier (Section 2.3.1). Possible filler gases that can produce light include neon, argon, krypton, xenon, and mercury. Mercury has been a gas of choice for many lamp manufacturers because it requires the least amount of excitation energy to produce UV radiation (Masschelein 2002). Mercury gas discharge lamps are generally classified according to the vapor pressure in the lamp. The four types of mercury arc lamps are low pressure (LP), low pressure high output (LPHO), medium pressure (MP) and high pressure. High pressure lamps are rarely used in water disinfection and will not be discussed any further.

2.4.1 LP and LPHO Lamps

LP lamps use mercury vapor. The vapor pressure in LP lamps is about 1 Pa (10^{-5} atm) (Masschelein 2002). LP UV lamps generally emit line radiation with a wavelength of 253.7 nm. This emission line is close to the maximum wavelength at which DNA and RNA absorb UV radiation. In addition, the temperature at the surface of the lamp is about 40°C. This temperature makes the LP lamps easier to work with, both in laboratory experimentation and in reactor design. The voltage fluctuation, the temperature of surrounding medium, and the aging of the lamps are several factors affecting the emission spectra of LP UV lamps (Masschelein 2002). LP UV lamps may have a lifetime of > 8 000 h (Bolton 1999).

LPHO lamps use an amalgam, which is an alloy of mercury using metals like gold, silver, copper and tin. The use of an amalgam in LPHO lamps offsets the self-absorption property of pure mercury gas at the low vapor pressure that LP lamps usually exhibit. Coating the inside of a LP UV lamp with another metal (e.g., indium and gallium) will allow the LP lamp to operate at a high power 2 to 3 times greater than that of LP lamps. LPHO lamps are growing in popularity among UV reactors designers because these lamps share most of the advantages of LP lamps but with a higher power output. LPHO lamps also have an average of > 8 000 h lifetime and a monochromatic spectrum whereas LP and LPHO UV lamps have 40 to 80 W and 100 to 200 W per lamp, respectively (Bolton 1999). LP UV lamps are easier to use in new disinfection studies because these lamps have fewer variables than MP lamps, and hence, the dose is easier to compute. In this present study, a LP lamp is used.

2.4.2 MP Lamps

Like the LP lamps, MP lamps use mercury vapor to produce broad-band polychromatic UV radiation with wavelengths ranging from 200 to 400 nm. The vapor pressure in MP lamps is in the 10^4 to 10^6 Pa range. The medium pressure lamps generally have a lower efficiency (10 to 15%) compared to LP lamps (35 to 40%). The power requirement for MP UV lamps (1 to 25 kW per lamp) needs to be considered when planning for new UV installations. MP UV lamps generate a higher linear output compared to LP and LPHO UV lamps, and hence, fewer lamps will be required for the same size of UV reactor having a LP or LPHO UV lamp. The additional heat generated by MP UV lamps causes a high rate of fouling of the lamp sleeve compared to LP lamps. Most MP UV lamps have an average of 3 000 to 5 000 h lifetime, which is shorter than that of LP lamps (>10 000 h).

2.4.3 Emerging Lamp Technologies

Interesting new developments in lamp technologies include the Xenon Flash and Excimer lamps. Flash lamps are blackbody emitters generating short pulses of about 1 to 30 pulses per s. The power requirement (1 to 5 kW per lamp) for flash lamps is between that of LP and MP lamps, but flash lamps have a very low efficiency (5 to 10%). Currently, the lifetime of flash lamps is less than 2 000 h, which is a limitation on applications. Excimer lamps, on the other hand, use a rare or halogen gas or a combination of different halogen gases. The efficiency of excimer lamps in producing germicidal UV radiation is about 8 to 10%, but a 25 kW power would be required per lamp. These new lamps attempt to compensate for the disadvantages of the mercury discharge lamp.

2.5 UV DISINFECTION OF DRINKING WATER

Several waterborne cryptosporidiosis outbreaks led to concerns about the effectiveness of free chlorine to inactivate *C. parvum* in drinking water. Prior to the implementation of the IESWTR, drinking water regulations had been developed to meet the inactivation of *G. lamblia*, bacteria and viruses by using free chlorine, monochloramine and ozone (USEPA 1999). One study reported that free chlorine was ineffective against *C. parvum* under drinking water treatment conditions (Korich et al. 1990). Earlier studies on the UV inactivation of *Cryptosporidium* oocysts reported high UV doses (Campbell et al. 1995; Lorenzo-Lorenzo et al. 1993; Ransome et al. 1993). In Lorenzo-Lorenzo et al. (1993), *Cryptosporidium* oocysts suspended in sterile demineralized water were exposed to UV radiation in a laminar airflow cabinet at an irradiance of 15 000 mW/s. Samples were placed 22 cm from the cabinet lamp and exposed for up to 180 min. Inactivation was measured with an infectivity mice assay. Due to ambiguity in the methodology and irradiance units, the UV dose could not be estimated, and hence, the data may have been misinterpreted. The authors achieved complete inactivation of 2.5×10^5 oocysts/mL (that is, > 5 log reduction) in 180 min. Ransome et al. (1993) reported that a UV dose of 120 mWs/cm^2 gave a 99% reduction in the viability of *Cryptosporidium* oocysts by using in-vitro excystation to measure inactivation. However, the study did not give a detailed description of the apparatus used to generate and expose the *C. parvum* oocysts to UV radiation. Therefore, little information about the effectiveness of UV disinfection could be obtained from this study. In another bench-scale study, *C. parvum* oocysts were captured and exposed to UV radiation on a 2 μm filter in a specially designed UV disinfection unit (Campbell et al. 1995). This study found that a dose of 8748 mWs/cm^2 from six LP lamps (three on each side of the filter) was needed to obtain > 2 log inactivation of *C. parvum* when in-vitro excystation and vital dyes were used to detect viability. The limitation of this study was that microorganisms in drinking water are most likely to be exposed to UV radiation suspended in water than caught on a filter.

However, the evidence that low doses of UV could be used to inactivate *C. parvum* meant that UV disinfection could be economically used to disinfect drinking water. Several studies that conducted collimated beam experiments by using an animal infectivity mouse model found that low UV doses could be used to inactivate *C. parvum* (Bukhari et al. 1999; Clancy et al. 1998; Craik et al. 2001). These studies reported that 8 to 20 mJ/cm^2 were able to achieve 4 to 6 log inactivation of *C. parvum*. This finding led to the development of UV regulations and the installation of many UV systems. In the proposed UV guidance manual, a UV dose ($I*t$) table was provided based on a statistical 90% confidence interval for the results of laboratory studies using relatively clean water samples. This table indicates that a dose of 12 and 11 mJ/cm^2 can be used to obtain a 3 log inactivation of *C. parvum* and *G. lamblia*, respectively. Moreover, *G. lamblia* exhibits a similar UV dose inactivation relationship with *C. parvum*. Nevertheless, UV reactors are typically designed to deliver a dose of about 40 to 60 mJ/cm^2 after all uncertainties and reduced equivalent dose biases have been taken into account (USEPA 2003b). However, a properly validated UV reactor with an extensive water quality data may be approved for UV of doses less that 40 mJ/cm^2. UV doses that could be economically delivered are considered to be low. Because a typical UV design dose is approximately 40 mJ/cm^2, UV doses less than this would be considered as low.

2.5.1 Advantages and Disadvantages of UV Disinfection

As an accepted alternative technology for water disinfection, UV has the following advantages and disadvantages over chemical disinfectants.

Advantages:

1. No known or potentially harmful DBP have been detected at the UV doses typically applied in drinking water.
2. UV radiation can readily inactivate *Cryptosporidium* oocysts, *Giardia* cysts and some viruses at UV doses easily achieved in drinking water treatment plants.
3. Temperature and pH have little effect on UV disinfection. However, low temperatures reduce the effectiveness of chlorination and ozonation.
4. A combination of UV and chlorine as primary and secondary disinfectants, respectively, will reduce the formation of chlorination DBP.

5. UV systems generally require a smaller footprint than other alternatives such as chlorination and ozonation.
6. Transportation and handling of hazardous chemicals is not required for UV disinfection.

Disadvantages:

1. Scattering and blocking of light by particles reduce the effectiveness of UV radiation to inactivate microorganisms.
2. No residual protection exists for treated water in distribution systems.
3. High UV doses are required to inactivate some viruses.
4. Determining the exact UV dose that microorganisms should receive in a UV reactor is difficult.
5. Some microorganisms might repair DNA damage with and without the presence of light.

2.5.2 UV dose determination

Bench-scale UV experiments usually use a collimated beam apparatus. This apparatus (described in Section 3.1.2) houses a UV lamp. A collimation tube is attached to the apparatus to produce a pseudo-parallel beam of UV radiation. Water samples placed in Petri dishes are exposed to incident UV radiation at a specific distance from the lamp. The incident UV irradiance is measured at the center point, C_p and averaged for the sample depth, d m (Figure 2-5).

2.5.2.1 Correction factors

The incident irradiance I (mW/cm^2) of the collimated beam is measured at the surface and center point, C_p. (Figure 2-5). Because the radiometer sensor, an incident UV radiation-measuring instrument, has a response spectrum, it weighs the different wavelengths in the UV range differently. For monochromatic light, the sensor factor is equal to 1. Light reflected from the surface of an irradiated sample must be accounted for even in a perfectly collimated beam. A reflection factor of 0.975 is applied to the radiometer reading to account for the reflection at the surface in accordance with

Fresnel's law, and a 2.5% reflection of the incident beam is assumed to occur (Bolton and Linden 2003). The Petri factor accounts for the radial distribution of the rays of light around the center of the beam. The radiometer sensor is usually smaller than the beam of light. Therefore, a ratio of the incremental measurements from the center of the beam in the x and y directions is divided by the measurement at the center point of the beam (Figure 2-5). A Petri factor that is > 0.90 depicts a good collimation of the rays (Bolton and Linden 2003).

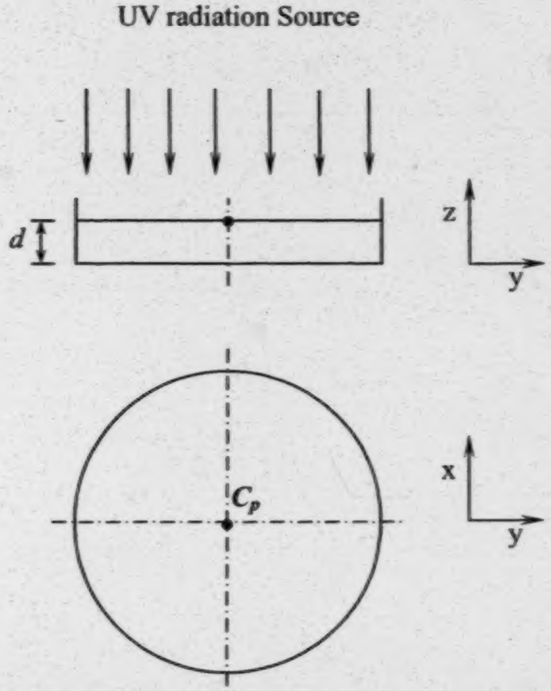

Figure 2-5 Schematic diagram of a Petri dish under a collimated beam of UV radiation. *d* is the sample depth and C_p is the center point.

2.5.2.2 Attenuation of UV radiation in water

When a sample of water is exposed to UV radiation, the incident irradiance is attenuated as the photons traveling through the water medium are absorbed by dissolved or undissolved compounds (Figure 2-5). Incident UV irradiance is the total radiant power incident on the surface of a sample from every UV direction (Bolton 1999). The absorbance of a liquid follows the Beer-Lambert law, which states that the absorbance of a liquid is directly related to the fraction of light attenuated as the light travels through the liquid. The attenuated UV radiation emerging after travelling a specific depth, *d* m, of the sample is the transmitted light. The transmittance, T_λ cm^{-1}, of the liquid at a specific wavelength can be described as

$$T_\lambda = \frac{E_\lambda^t}{E_\lambda^o}, \qquad \text{Equation 2-2}$$

where E_λ^t and E_λ^o are the transmitted and incident light irradiance while λ is wavelength in nm. The transmittance is related to the absorbance of the liquid by

$$T_\lambda = 10^{-a_\lambda l}, \qquad \text{Equation 2-3}$$

where *a* is the absorption coefficient (cm^{-1}), and l is the path length (m) (Bolton 1999). The integrated form of the Beer-Lambert law is used to account for the attenuation of UV radiation in water samples:

$$\text{Water Factor} = \frac{1-10^{-al}}{al \times \ln(10)}, \qquad \text{Equation 2-4}$$

where *a* is the absorption coefficient (cm^{-1}) and l is the path length (cm) (Bolton and Linden 2003). The beam diverges as it travels through the liquid. This divergence reduces the average irradiance in the sample. To account for this difference, a divergence factor is estimated from

$$\text{Divergence Factor} = \frac{L_d}{L_d + l}, \qquad \text{Equation 2-5}$$

which is the integral form of $1/L_d^2$ which describes the rate at which the irradiance drops due to the divergence of the beam. L_d (mm) is the distance from the surface of the lamp to the top of the sample, and l (mm) is the path length (Bolton and Linden 2003).

The depth-averaged germicidal UV irradiance, I_{avg} (mW/cm^2), is then estimated from

I_{avg} = Incident irradiance, *I* (mW/cm^2) * Petri factor * reflection factor * water factor * divergence factor Equation 2-6

The depth-averaged UV irradiance represents a measure of the effective UV radiation that will be available for inactivating microorganisms in a sample volume (Morowitz 1950).

2.5.2.3 Calculation of UV dose

For an irradiated sample, the UV dose can be computed as

$$D = I_{avg} * t, \qquad \text{Equation 2-7}$$

where D = Calculated UV dose (mJ/cm^2)

I_{avg} = Depth averaged UV irradiance (mW/cm^2)

t = Exposure time (s)

2.5.3 Theories of disinfection

Chick (Chick 1908) observed that the rate of inactivation of microorganisms ($-\frac{dN}{dt}$) was proportional to the microorganisms concentration, N:

$$-\frac{dN}{dt} = kN, \qquad \text{Equation 2-8}$$

where *k* is the first-order rate constant, N is the number of survivors, and t is time. The integrated form of Equation 2-8 was later found to have a close analogy to a pseudo first-order chemical reaction (Equation 2-9):

$$-\ln\frac{N_f}{N_i} = kt, \qquad \text{Equation 2-9}$$

where N_i and N_f are the initial and final number of microorganisms after time, *t*.

A modified Chick's law proposed by Watson (Watson 1908) can be represented as

$$-\ln\frac{N_f}{N_i} = kC^{n_d}t, \qquad \text{Equation 2-10}$$

where C is the concentration of the disinfectant, and n_d is the dilution coefficient. However, n_d is often considered to be equal to 1. To apply equation 2.10 to UV disinfection, the concentration of the disinfectant is replaced the by irradiance:

$$-\ln\frac{N_f}{N_i} = kIt, \qquad \text{Equation 2-11}$$

where I is the irradiance (mW/cm^2), and It is the UV dose (mJ/cm^2).

In the literature, two theories of disinfection, the vitalistic and mechanistic theories, have been discussed. The proponents of the vitalistic theory believe that the resistance of the individuals in a population to a disinfectant is unequal. This gradient of resistance ranging from little-or-no-resistance to highly resistant microorganisms is referred to as the "theory of variable permanent resistance" (Lee and Gilbert 1918). This phenomenon is believed to be responsible for the "shoulder" and "tailing" effects observed with inactivation curves, which describe the relationship between the proportion of inactivated microorganisms and the UV dose (USEPA 2003b). As a result of this resistance, individuals in a population of microorganisms is believed to be inactivated at different rates depicting the shoulder, linear rate and tailing sections of the inactivation curve. In accordance with the mechanistic theory, Equation 2-11 has been used by many researchers (Amoah et al. 2005; Batch et al. 2004; Blatchley et al. 2001; Craik et al. 2002; Jolis et al. 2001; Loge et al. 1996; Mamane-Gravetz and Linden 2004; Mamane-Gravetz and Linden 2005; Qualls and Johnson 1983; Qualls et al. 1985; Severin et al. 1983a; Severin et al. 1983b; Sommer et al. 1995; Sommer et al. 1998) to present the results of their UV inactivation studies on a semi-log graph. Under ideal conditions, the relationship between the log survival ratio and the UV dose is expected to be linear. However, in reality, we observe other relationships which include a linear section in addition to the "shoulders" and "tails" depicted by curves A, B and D in Figure 2-6.

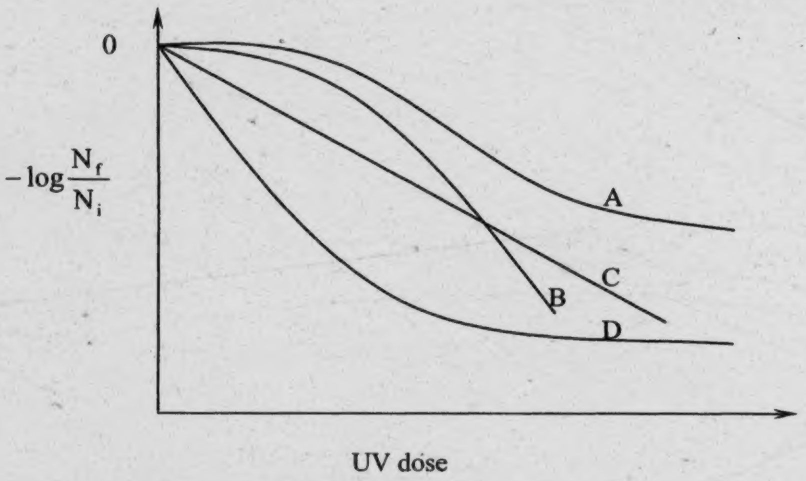

Figure 2-6 Types of microbial inactivation or survival curves exhibiting: shoulder and tailing (A), shoulder (B) linear rate (C) and tailing (D).

2.6 UV INACTIVATION OF *B. SUBTILIS*

2.6.1 Surrogate Microorganisms

Several microorganisms have been identified for their suitability as surrogates for pathogens in drinking water. The concentration of pathogens in raw water is usually too low for them to be isolated and identified easily. Therefore, the use of surrogate microorganisms in drinking water disinfection studies becomes necessary. For a microorganism to be suitable for use as a surrogate, it should possess the following characteristics:

1. The surrogate should be inexpensive, non-pathogenic and easy to culture in the laboratory in large numbers;
2. The surrogate should be of equal or greater resistance than the pathogenic microorganisms of interest;
3. The UV dose response of the surrogate should be well-defined and reproducible;
4. Surrogates should be reliably detected at low numbers, and
5. Surrogates should not replicate in water.

Because one of the main tasks of this study was to produce surrogates that break through the filtration process, it was useful to have a microorganism that was smaller in size than the pathogenic microorganisms, *C. parvum*, that could form a cyst or spore but meet the characteristics of a suitable surrogate. Two common surrogates used in drinking water research are *B. subtilis* and MS2 coliphage. However, only the *B. subtilis* can form a spore of these two microorganisms. More importantly, the *B. subtilis* spore (1.2 to 1.5 μm) was in the size range as *C. parvum* oocysts (3 to 5 μm) compared to that of viral particles (0.01 to 0.025 μm). Consequently, the *B. subtilis* was chosen as the surrogate for this study.

2.6.2 Biology of *B. subtilis*

The genus *Bacillus* is a member of the family *Bacillaceae*, which are mostly aerobic and facultative bacteria. The characteristics of the family include rod-shaped cells, the formation of endospores, being gram-positive, and mobility by lateral flagella (Maruo and Yoshikawa 1989). Spore-forming microorganisms form spores when challenged by a lack of nutrients or other environmental stresses including heat. The spore coat, which protects the spore from chemical agents and many other environmental stresses including dehydration, is made of more than 25 highly cross-linked polypeptides species. However, this tough outer protective coat does not prevent the spore coat from responding to favorable germination conditions (Driks 1999). Most research involving drinking water and bacterial spores has used *B. subtilis* (Chang et al. 1985; Harris et al. 1987; Qualls et al. 1989; Qualls and Johnson 1983; Rice et al. 1996; Rice and Ewell 2001; Sommer and Cabaj 1993; Sommer et al. 1995; Sommer et al. 1998; Uvbiama and Craik 2005). Figure 2.7 shows an electron microscopic image of *B. subtilis* spores.

Figure 2-7 Scanning electron microscope (SEM) image of *B. subtilis* captured on a 0.4 μm polycarbonate track etched filter (generated as part of this study).

The spores were ellipsoidal in shape with average dimensions approximately 1.5 μm long and 0.7 μm in diameter. The pasteurization of spore suspensions is one way of eliminating vegetative bacteria and ensuring that only spores are present. The heat resistance of a spore depends on the state of dehydration of the spore core whereas the susceptibility of the spores to UV inactivation depends on the small-soluble proteins bound to the chromosome (Driks 1999).

2.6.3 Dose Response of *B. subtilis* spores

B. subtilis spores have been used in several collimated beam studies under different growth and exposure conditions. Figure 2-8, reveals that the inactivation curves exhibit a *shoulder* similar to the curves shown in Figure 2-6. Harm (1980) reported that shoulders are usually characteristic of double-stranded DNA. Based on information collected from the literature, the average UV dose at specific inactivation levels was computed. A UV dose of approximately 22, 34, 42 and 48 mJ/cm^2 will be able to achieve 1, 2, 3 and 4 log inactivation of *B. subtilis*, respectively. The dose response of spores to UV radiation is affected by the age, density, sporulation medium, lysozome sensitivity and heterogeneity of the DNA (Cerf 1977). Table 2-1 provides details of the different sporulation media and water matrices used by the different researchers.

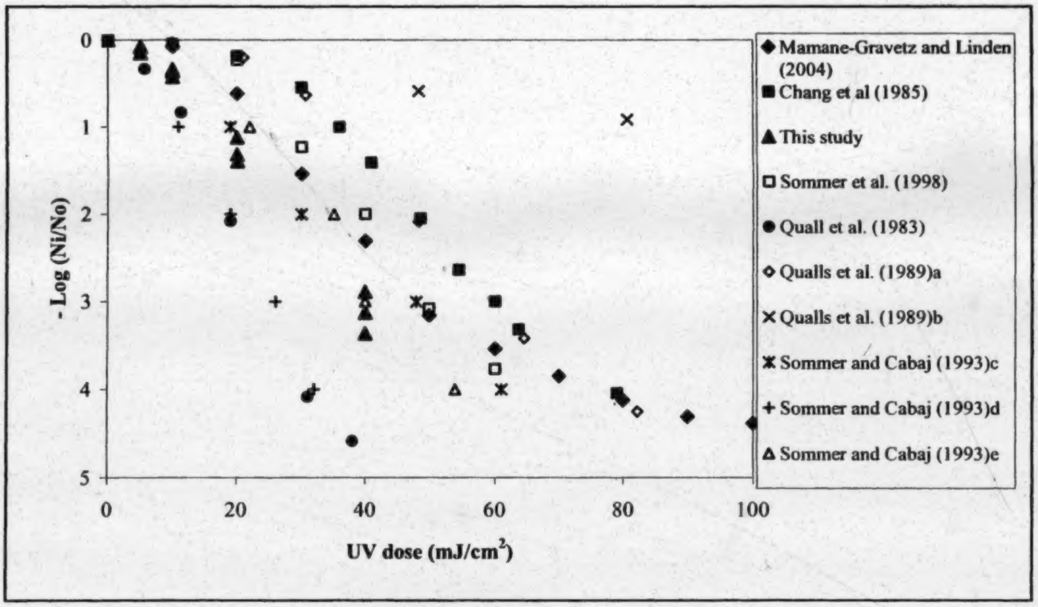

Figure 2-8 Dose response curves of *B. subtilis* spores obtained from published peer-reviewed studies compared to the dose response of the *B. subtilis* spores used in this study. The designations (a, b) and (c, d, e) indicate that the data is from the same study, but growth conditions and UV exposure procedures are different (Table 2.1).

2.7 UV INACTIVATION CURVE MODELING

The target theory, which is closely related to the second law of photochemistry, is important in understanding the modeling of inactivation curves of spores. The target theory states that a finite number of targets (sites) are on every individual cell (or spore) and that these targets are vulnerable to randomly distributed hits (Harm 1980). Under the one-hit one-target theory, one hit is assumed to inactivate a cell. As a result, the inactivation curve should be linear and follow the Chick-Watson law. This result has been observed to be the case for most single-stranded DNA and RNA in viruses (Harm 1980). However, in microorganisms with double-stranded DNA and RNA, several targets, *n*, require one hit (multi-target) or one target requires a series of hits, *n*, (multi-hit or series events) before the microorganisms are inactivated. Hence, the multi-target and multi-hit models describe the shoulder phenomenon better than the one-target one-hit model.

Table 2-1 Growth media and water matrices used in the collimated beam studies shown in Figure 2.6.

Sporulation media	Water matrix	Source
Schaeffer's broth	Filtered wastewater	(Chang et al. 1985)
N/A	Filtered water	(Mamane-Gravetz and Linden 2004)
N/A	Buffered water	(Qualls and Johnson 1983)
[a] Schaeffer's medium	Buffered DI water	(Qualls et al. 1989)
[b] BBL AK agar		
Liquid Enrichment medium	Distilled water	(Sommer et al. 1998)
[c] Columbia agar	Potable water	(Sommer and Cabaj 1993)
[d] Schaeffer's agar		
[e] Schaeffer's broth		
Schaeffer's broth	Phosphate buffered DI Water	(Uvbiama and Craik 2005)

N/A – Not Available

[a, b, c, d, e] Distinguishes the sporulation media used in the same study as shown in Figure 2.8

The multi-target and multi-hit models are given in equation 2-12 and 2-13, respectively. The multi-target is given by

$$\frac{N_{fi}}{N_i} = 1 - (1 - e^{k_m It})^{n_c} ,$$

Equation 2-12

and the multi-hit (series event) is given by

$$N_f = N_i e^{-k_s It} \sum_{i=0}^{n-1} \frac{(k_s It)^{n_c}}{n!}, \qquad \text{Equation 2-13}$$

where N_f and N_i are the final and initial number of microorganisms, k_m and k_s are the first-order rate constants (cm^2/mJ) for the multi-target and series event models, I is the irradiance (mJ/cm^2), t is time (s), and n_c is the number of hits or targets for the multi-hit and multi-target model, respectively.

The multi-target and multi-hit models explain the lag phase (shoulder) in inactivation curves. When the linear section of an inactivation curve is extended to the ordinate, it gives a measure of the number of targets, n, for multi-target modeled data or the number of hits, n_c, for multi-hit modeled data.

2.8 EFFECT OF PARTICULATE MATTER ON UV INACTIVATION OF MICROORGANISMS

2.8.1 Light Scattering in Water

A wide variety of particles are present in natural water, including inorganic, living or dead organic matter and biotic materials (USEPA 1999). These particles can also be classified in terms of origin, concentration, size and surface chemistry (AWWA 1999). Figure 2-9 presents a spectrum of particles found in natural water. Particles that are less than 50 μm in size usually remain suspended in water (Van Gilder et al. 1999). These particles include inorganic clays, silts, pathogenic and non-pathogenic microorganisms, asbestos fibers, terrestrial detritus, and waste-discharge constituents that are generated from land-based and atmospheric sources (AWWA 1999).

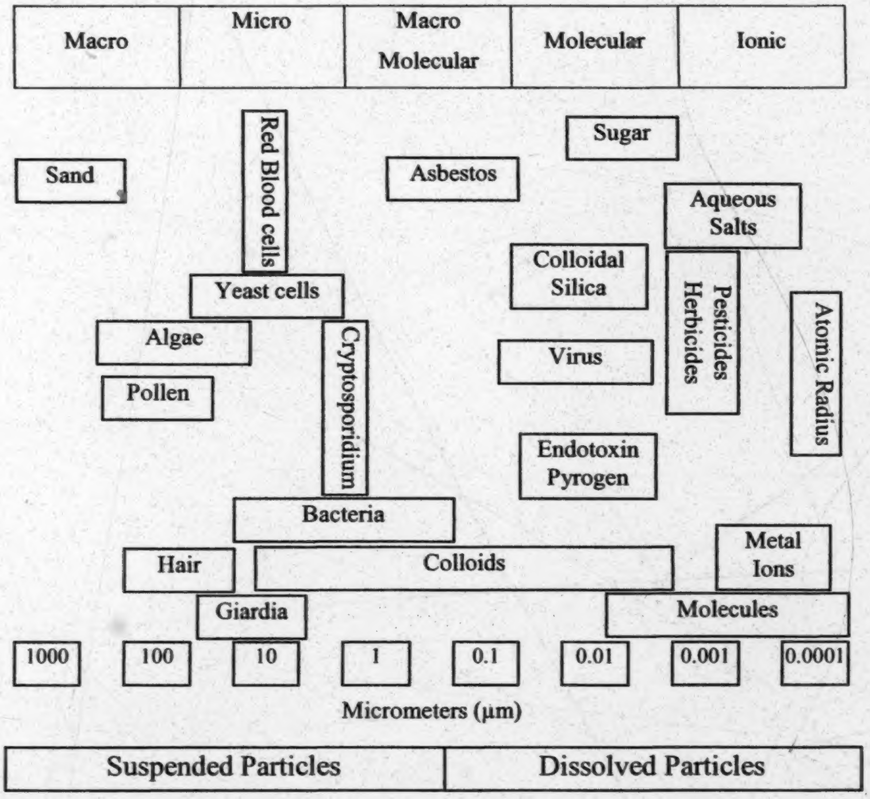

Figure 2-9 Spectrum of particles commonly found in natural water in correlation to their sizes (USEPA 1999).

The effectiveness of UV radiation to inactivate microorganisms is reduced by the presence of particles in water. When light traveling in a straight path encounters an obstacle, the photons are either absorbed, scattered (forward and backward) or both (Figure 2-10). Figure 2-10 reveals the possibility that the light can be blocked from reaching microorganisms attached to the particles. The factors that contribute to the intensity of the scattered light include the particle concentration, diameter of the particle, measuring angle, wavelength, and refractive index of the particles in relation to the surrounding water medium (Huber and Frost 1998).

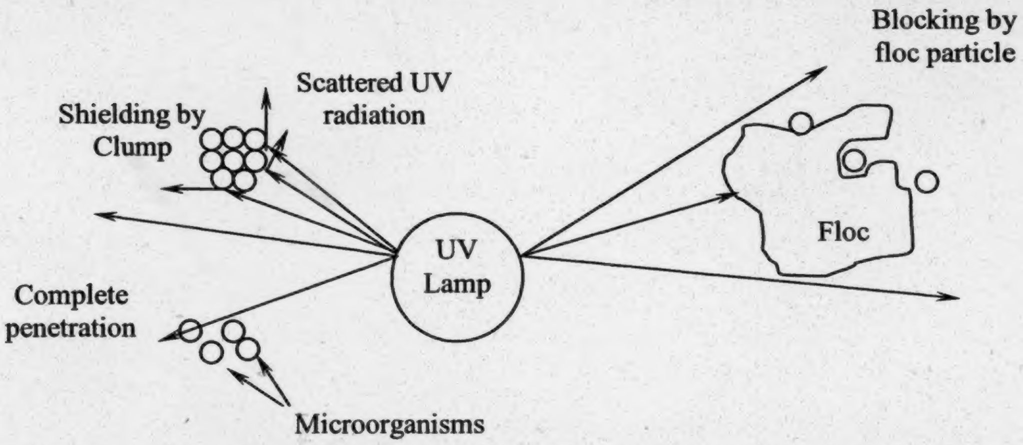

Figure 2-10 Blocking, scattering and absorption of UV radiation by particles in water. Adapted from USEPA (1999)

The presence of suspended particles in filtered and unfiltered drinking water may have an impact on UV dose. Particles may increase the absorbance of UV photons within the irradiated water, thus reducing the effective UV dose, as described previously. One study found that the UV dose was underestimated when the UV dose calculations were based on conventional transmission spectrophotometry of unfiltered water (Christensen and Linden 2003). In order to account for the difference, a spectrophotometer with an integrating sphere attachment was suggested to provide a more accurate measurement of the true depth-averaged UV irradiance. However, the authors considered only the effect of particles on UV dose within a water sample and not the effect of microorganism-particle interactions on inactivation.

Clay particles are ubiquitous in natural water. Several studies have investigated the effect of clay particles on UV inactivation (Bitten et al. 1972; Assenting et al. 2004; Templeton et al. 2005). Qualls et al (1983) indicated that clay particles may have an insignificant effect on the UV inactivation of microorganisms because these particles tend to scatter light more than they adsorb it. Scattered light may still be available to inactivate microorganisms. This finding might be one of the reasons that Assenting et al. (2004) did not find any significant difference in the UV inactivation of seeded MS2 bacteriophage in a collimated beam experiment in which the effect of seeded particles (clay and algae) on suspension absorbance was taken into account in the dose determination. Templeton et al.

(2005) studied the protective effect of particles (kaolin clay, humic acid and activated sludge particles) suspended in synthetic water on the UV inactivation of viral surrogates (MS2 coliphage and bacteriophage T4) by using alum and ferric chloride coagulants under two filtration conditions (none and 0.45 μm). In that study, it was found that there was no protective effect of clay particles on the viral surrogates under both filtration conditions when samples were exposed to 40 and 80 mJ/cm^2. This result is similar to those reported by Bitton et al. (1972) and Passantino et al. (2004).

The particles in secondary municipal wastewater are mostly organic materials that have co-existed with indicator microorganisms like coliform bacteria. Coliform bacteria have been observed to be attached to or be enmeshed by particles in wastewater. This finding has been the basis of many studies on particle-association using wastewater.

2.8.2 The Effects of Particle-association on UV Inactivation

The effects of particle-association on UV disinfection have been studied mainly using coliform bacteria in wastewater (Emerick et al. 2000; Emerick et al. 1999; Jolis et al. 2001; Loge et al. 2001; Loge et al. 1996; Loge et al. 1999; Qualls et al. 1983; Qualls et al. 1985). In one wastewater disinfection study, suspended particles led to a significant reduction of the UV inactivation rate of coliform bacteria between filtered and unfiltered secondary effluent (Qualls et al. 1983). The inability to achieve a 4 log reduction of coliform bacteria in unfiltered effluent at a UV dose of 16 mJ/cm^2 was attributed to the shielding of some of the coliform bacteria by wastewater bacterial floc particles.

The isolation and removal of particles that may harbor microorganisms have improved the inactivation rate of microorganisms in unfiltered water. Liltved and Cripps (1999) found a 3 log difference in inactivation between unfiltered and filtered seawater containing *Artemia* fragments. Because a 3 log reduction was required for fish ponds, the authors observed that filtering samples through a 355 μm, 80 μm, and 50 μm improved the log reduction of *Artemia* fragments by approximately 1.3, 2.3 and 3.0 log, respectively, when a UV dose of 22 mJ/cm^2 was applied (Liltved and Cripps 1999).

The size of the particles in water is one of the factors that affect the ability of particles to shield and/or engulf microorganisms. Qualls et al. (1983) filtered secondary effluent through an 8 and 70 µm membrane filters and assumed that coliform bacteria in the effluent would be dispersed when filtered through an 8 µm filter. Although filtration through a 70 µm filter improved inactivation compared to the unfiltered samples, tailing of the inactivation curve was still evident. This finding implies that the microorganisms in the filtered effluent were still associated with particles larger than 70 µm. However, after an 8 µm filtration, the inactivation curve did not show any significant tailing at the UV dose studied (Qualls et al. 1983). Therefore, a minimum particle-size needs to be present for shielding to occur. Liltved and Cripps (1999) studied a minimum filter size of 50 µm, but recorded a significant increase in the inactivation rate for filtered samples compared to unfiltered samples. However, the survival curves showed that some tailing was measured at the filter sizes and UV dose studied. This finding means that *Artemia* fragments may still have been attached to particles ≤ 50 µm. In separate studies, particles in the 7 to 10 µm size range or smaller had little effect on the UV inactivation of coliform bacteria in secondary effluents (Emerick et al. 2000; Jolis et al. 2001). In Templeton et al. (2005), humic acid and activated sludge particles had significant protective shielding effect on the UV inactivation of MS2 coliphage and bacteriophage T4 even though water samples were filtered through a 0.45 µm membrane filter. This study was able to demonstrate that the size of particles that shielded the viral surrogates might have been smaller than 0.45 µm.

In a multiple barrier water treatment system, disinfection is typically the final barrier against microorganisms not removed by filtration. However, in cases where the coagulation is poor, filtration may be sub-optimal and fail to meet desired standards, and turbidity excursions might occur. In one study, a prepared microbial cocktail containing *C. parvum* oocysts, *Encephalitozoon instestinalis* spores, enteropathogenic *Escherichia coli* O157:H7, *Aeromonas hydrophila*, and MS2 bacteriophage were seeded into river water (Harrington et al. 2003). All the microorganisms tested were detected in the filtered water of a pilot-scale dual-media filter when there was turbidity break through occurred. Although concerns about the state of aggregations of break through microorganisms were raised in this study, no further investigation was initiated. Both particles and microorganisms can be expected to be present in poorly filtered and unfiltered water. In many cases, their presence has caused waterborne outbreaks (Hrudey and Hrudey 2004). Therefore, UV reactors can be reasonably expected to be challenged by microorganisms that have broken through a filter media during periods of sub-optimal coagulation.

Studies on particle-association using filtered and unfiltered water have produced different results. For example, Passantino et al. (2004) and Batch et al. (2004) also found that the presence of particles did not have a significant effect on the UV inactivation of microorganisms. Batch et al. (2004) reported that UV radiation was effective for inactivating seeded MS2 coliphage into filtered drinking water that met federal regulations when UV dose measurements took turbidity, particle count and absorbance into consideration. In contrast, Amoah et al. (2005) found that an increase in the concentration of biological and non-biological particles within a 5 to 25 μm size range that were concentrated from the bottom sediment of Lake Okanagan, British Columbia, Canada led to a statistically significant but modest reduction in the inactivation of seeded with *C. parvum* oocysts and *G. muris* as turbidity increased from 0.3 to 20 NTU and 7.5 to 20 NTU, respectively.

2.9 Need for This Research

UV reactors are increasingly being used in the drinking water industry. More people will depend on these reactors to provide safe drinking water. Particle-association of microorganisms needs to be accounted for in UV reactors design and validation because studies have shown that UV disinfection is affected by the presence of particles in water, especially if the microorganisms are completely engulfed by the particle or floc material. The current USEPA *I*t* table is generated with an underlying assumption that microorganisms will be dispersed. This assumption implies that the unit operations and processes of multi-barrier treatment systems are independent of one another. However, the effectiveness of UV disinfection will be affected if, for instance, the coagulation or flocculation process fails.

Seeding microorganisms and particles into relatively clean water is the method most often used to investigate the effects of particles on the UV inactivation of microorganisms in drinking water. However, such methods have several drawbacks. Elevated amount of seeded particles in filtered, unfiltered or synthetic water does not represent the raw water those studies aim to investigate. In addition, natural and artificial particles may have different scattering and absorption properties, which may affect the refractive index of the water matrix being studied. Furthermore, aggregation may not be accounted for in seeding studies that add microorganisms to filtered or unfiltered water. In water treatment systems, coagulation and flocculation promote the aggregation of microorganisms in raw water. Hence, the UV inactivation of microorganisms seeded into relatively clean water may not represent the UV inactivation of the microorganisms that break through media filters.

Aggregation is usually not accounted for in the determination of the effects of particle-association on UV inactivation. Drinking water treatment involves a series of processes that promote aggregation but have been unaccounted for in many laboratory studies to date. This present study will attempt to not only account for the effect of upstream treatment processes, but will use naturally occurring particles. Consequently, this study will be unique compared to most past studies on particle-association. Another aspect of this study worthy of mention is that allowing the microorganisms to break through a sand filter creates a unique set of particles. One study used floc materials that were "feathery" and weak (Templeton et al. 2005) but this present study uses floc materials that have gone through the interstices of a sand/anthracite media filter. Travelling through the interstices shaved off the weak parts of the floc. This procedure gives the break through particles and microorganisms a unique scattering intensity and absorbing properties that will be close to what pilot or full-scale systems may produce. A comparison of the UV inactivation of spores that were seeded into filtered water and the UV inactivation of spores that broke through a media filter was conducted.

3 EXPERIMENTAL MATERIALS AND METHODS

This chapter details the experimental materials and methods used in this study. The experiments in this thesis were conducted in two phases. Phase 1 experiments (preliminary) were conducted in November 2004 whereas the Phase 2 experiments were conducted in August 2005. The difference in the experimental materials and methods between the two phases is provided only in sections where a different material, method or technique was used. This chapter is divided into nine sections: equipment, raw water collection and preparation, *B. subtilis* spores methods, simulation of upstream processes, filtration, UV exposure, measurement of particle-association by serial filtration, and electron microscopy. Unless otherwise stated, sections with no reference to either phase imply that the material or method described in that section is either common to both phases or refers to Phase 2 experiments.

3.1 EQUIPMENT

3.1.1 Homogenizer

The objective of the homogenization step was to break up aggregates and disperse spores attached to or embedded within aggregates. The effect of aggregation on spore inactivation was determined by comparing the UV inactivation of spores homogenized after UV exposure to the UV inactivation of spores homogenized prior to UV exposure. Inactivation was also compared to that of spore preparations suspended in phosphate buffered de-ionized (DI) water. The PowerGen 700 (Model 700, Fisher Scientific, Pittsburgh, PA) is a variable speed (10 000 to 30 000 rpm) post-mounted homogenizer (Figure 3.1). The external diameter of the homogenizer shaft is 7 mm. The homogenizer shaft is protected by the generator tube. A knife (6 mm diameter and 12.7 mm long) is attached to one end of the shaft. The rotation of the shaft and knife at high speed in liquid performs the homogenizing operation. The particles and clumps were probably broken down by the shearing action created by the high-speed bubbles emanating from the perforated end of the generator tube housing the knife.

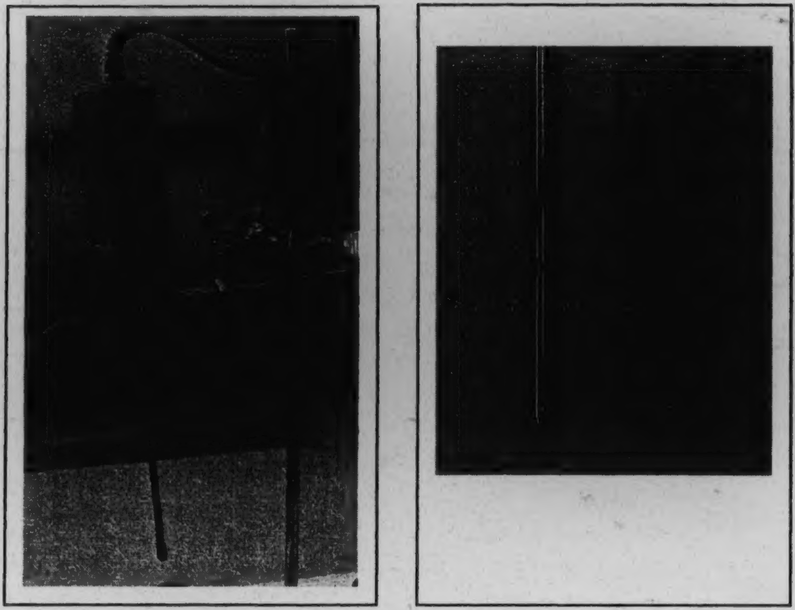

Figure 3-1 Fisher Scientific PowerGen 700 Homogenizer

3.1.2 Collimated Beam Apparatus

A collimated beam apparatus (Calgon Carbon Corporation, USA) housing a 10 W LP mercury arc lamp (Ster-L-Ray Germicidal Lamp, model G12T6L 15114, Atlantic Ultraviolet Corporation, Hawpange, NY) was used for all UV exposures. The unit uses an gas-operated pneumatic shutter, and the lamp chamber is air-cooled by a fan. The UV exposures were timed with a stop watch at the opening and closing of the shutter. A collimation tube (183 mm long and 58 mm ID) was used for the UV exposures. The distance between the lamp and the surface of the liquid sample was 460 mm while the distance between the end of the collimation tube and the sample surface was 30 mm. The distance between the lamp and the sample surface was adjusted with a laboratory jack.

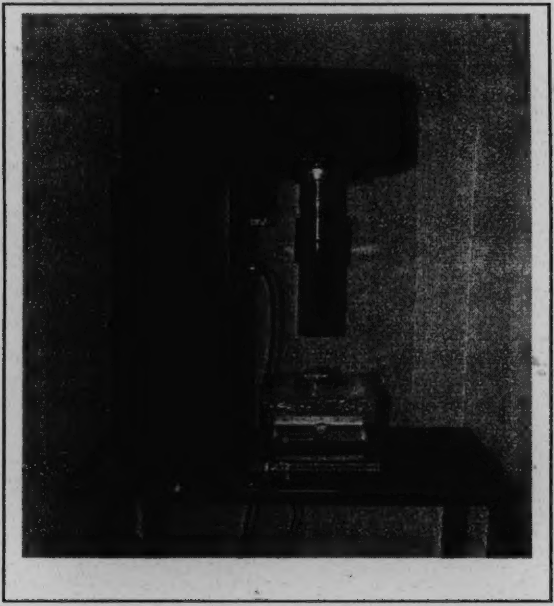

Figure 3-2 Collimated Beam apparatus fitted with a Ster-L-Ray low pressure mercury arc lamp. Samples are continuously stirred during UV exposure.

3.2 RAW WATER SAMPLE COLLECTION AND PREPARATION

3.2.1 Rossdale Water Treatment Plant

The Rossdale water treatment plant is one of two drinking water treatment plants that serve the greater Edmonton area, Alberta, Canada. The plant's water intake is located in the North Saskatchewan River. The plant, which has a production capacity of 450 ML/d, employs coagulation, flocculation, settling and filtration trains to treat the water. Disinfection is carried out by three parallel UV reactors followed by monochloramine.

3.2.2 Sample Collection and Preparation

River water samples from the North Saskatchewan River were collected at the Rossdale water treatment plant. Samples were collected in 20 L plastic containers in accordance with *Standard Methods* (Easton et al. 1995). To verify that the number of indigenous aerobic spores present in the raw water was low, sub-samples of 25 mL of the raw water were dispensed into sterilized test tubes, placed in a water bath, heated to 80°C for 15 min, and enumerated as described in Section 3.4.2. A high concentration of indigenous spores could interfere with the experimental results because indigenous spores have been shown to be more resistant to UV than laboratory-prepared spores (Mamane-Gravetz and Linden 2004). Water samples were stored at 4°C until their use in experiments. Table 3-1 shows the river water data used for this study.

Table 3-1 Raw water samples collected from the Rossdale Water Treatment Plant in September 2004 (Phase 1) and July 2005 (Phase 2). Data provided by EPCOR Water Services.

Water Parameter	Phase 1	Phase 2
Turbidity, NTU	4.8	28.8
pH	8.3	8.3
Conductivity, µS/cm	ND	356
Total hardness, mg/L	ND	176
Alkalinity, mg/L	130	134
Color, TCU	ND	13

ND – Not done

3.3 *B. SUBTILIS* METHODS

3.3.1 Production

Stock suspensions of *B. subtilis* (ATCC 6633, American Type Culture Collection, Manassas, VA.) were produced by using the modified Schaeffer medium method (Leighton and Doi 1971) with some modifications. Initially, the pre-culture was prepared from freeze-dried spores added to 8 g nutrient broth/L (BBL Nutrient Broth, Benton Dickinson Microbiology Systems, Cockeysville MD). The broth was incubated at 37°C on an incubator shaker (Innova 4080, New Brunswick Instruments Co. Inc., Edison, NJ) at 180 rpm for 24 h. Subsequently, pre-cultures for other batches were generated from isolated *B. subtilis* colonies from streak plates. The growth medium was prepared by adding 0.1 mL/100 mL of a filter-sterilized (0.22 µm Millex® GS Filter unit, Millipore, Cork Ireland) solution of 1 µM $FeSO_4$, 10 µM $MnCl_2$, and 1 mM $CaCl_2$ to sterile nutrient broth (sterilized at 121°C for 15 min). After gentle swirling, the mixture was inoculated with the *B. subtilis* pre-culture and incubated at 37°C on an incubator shaker at 180 rpm for 72 h. On completing sporulation, *B. subtilis* spores were harvested by centrifugation at 7500 × g for 20 min at 4°C. The supernatant was decanted and the pellet re-suspended in sterile DI water. This wash step was done three times. Once complete, spores were pasteurized in a water bath at 80°C for 30 min with intermittent swirling of the solution. The stock solution was finally re-suspended in 50% ethanol, homogenized at 30 000 rpm for 3 min, and stored at 4°C. The concentration of spores in the stock solutions used for this study was ~ 1.5×10^9 CFU/mL. The presence of spores in the stock solution was verified by using Schaeffer – Fulton staining and phase-contrast microscopic examination.

3.3.2 Spore Enumeration

Spores in the stock preparations and experimental samples were enumerated by using a pour plate technique. A decimal dilution series of the stock or sample was prepared. An aliquot (1 mL) of stock or diluted sample was added to 9 mL of sterile DI water, and the suspension was vortex mixed (Genie 2 Fisher Vortex, Fisher Scientific, Bohemia, NY) for about 30 s to 1 min at a medium setting. One milliliter of this suspension was transferred aseptically to a sterile 150 x 15 mm sterile polystyrene Petri dish (Fisherbrand). Approximately 15 mL of nutrient agar made from 8 g nutrient broth/L and 16 g solidifying agar/L (Difco Laboratories Detroit, MI) was poured onto the 1 mL suspension. The contents of the dish were gently but thoroughly mixed by using back-and-forth and swirling motions. Solidified agar plates were incubated at 37°C for 48 h. The colony forming units (CFU) was counted with the aid of a colony counter (Quebec Colony Counter, Buffalo NY). All plating and enumerations were done in triplicate.

3.3.3 Impact of Homogenization on the Viability of *B. subtilis* Spores

The objective of this experiment was to determine the effect of the homogenization step on the viability of spores. To measure this effect, three clean and sterile flasks (Flasks A, B and C) containing 100 mL of sterile DI water were seeded with *B. subtilis* spores to a concentration of 6.4 x 10^7 spores/mL. Flask A was homogenized once (PowerGen 700) while Flask B was not homogenized. Flask C was homogenized daily for 3 days to determine whether repeated homogenizing had any effects on viability. In addition, Flask C was aimed at determining whether the speed and time setting were optimal. An increase in colony counts over time would indicate that the homogenization step was incomplete. Each day, two 10 mL sub-samples were taken from each flask. Every sub-sample was homogenized at 30 000 rpm for 30 s before enumeration. This experiment was conducted at 0, 24, 48 and 72 h.

3.4 SIMULATION OF UPSTREAM TREATMENT PROCESSES

A jar test apparatus (PB-700^{TM} Jar Tester, Phipps & Bird, Richmond, VA) was used to simulate the upstream water treatment processes (coagulation, flocculation and settling). The jar testing protocol used for this study was adapted from the one used by EPCOR Water Services operators at the Rossdale water treatment plant. Each of the six square jars was filled with 2 L of raw river water. Each jar was seeded with *B. subtilis* spores to approximately 10^6 CFU/mL. The contents were then mixed at ~ 75 rpm for about 30 min. Mixing was increased to 300 rpm, and 9 mg alum/L (Cleartech, Edmonton, AB) was added simultaneously. This alum dose was 60% of the optimal dose determined for this water in jar tests. After 60 s of rapid mixing, the speed was reduced to 75 rpm, and 0.5 mg Percol LT27A anionic polymer/L (Allied Colloids Inc., Brampton, ON) was added immediately. After 3 min, the speed was reduced to 50 rpm for another 3 min, and finally to 25 rpm for 3 min. The water was allowed to settle for an additional 3 min. The settled water from the six jars was transferred to a sterile 20 L reservoir, which was connected to a laboratory-scale dual media filter column. The average turbidity of the settled water was measured on a Model 965 Turbidimeter (Orbeco Hellige Turbidimeters, Orbeco Analytical Systems, Farmingdale, NY). Particle size and concentration were measured by using a Hiac-Royco Particle Counting System (HRLD-150 Sensor, Model

8000A Particle Counter and Automatic Bottle Sampler). The cumulative and differential particle counts for ≥ 2, ≥ 5, ≥ 7, ≥10, ≥ 14, ≥ 20, ≥ 40, and ≥ 80 μm were reported by the particle counter. Data of four replicated measurements of particle counts found in the filtered water are presented in Figure 4-15.

3.5 FILTRATION

Figure 3.3 shows a schematic diagram of the bench-scale 2.5 cm ID filter column used for this study. Because the primary purpose of the experiment was to simulate the aggregation of microorganisms that may be encountered in conventional drinking water treatment systems and was not to assess the performance of the filter, the use of a 2.54 cm ID filter column was acceptable. The filter was designed and operated in a constant-head declining rate mode. A 100 cm long clear PVC pipe was filled with 20 and 7.2 cm of crushed quartzite filter sand and anthracite filter grade coal, respectively (AWI Filters, Calgary AB).

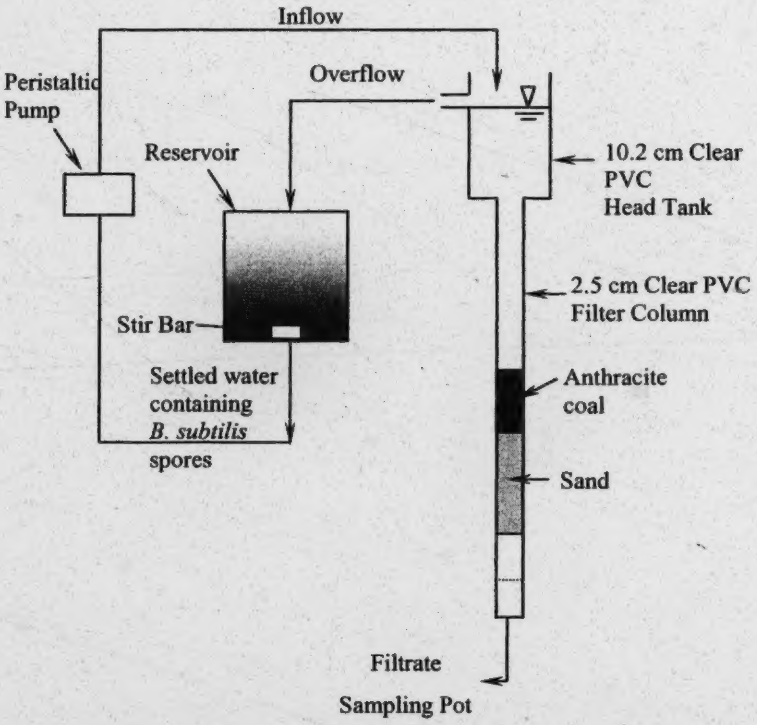
Inflow
Peristaltic Pump
Overflow
Reservoir
10.2 cm Clear PVC Head Tank
2.5 cm Clear PVC Filter Column
Stir Bar
Settled water containing *B. subtilis* spores
Anthracite coal
Sand
Filtrate
Sampling Pot

Figure 3-3 Schematic diagram of the laboratory-scale dual-media filter column.

Table 3-2 provides the physical properties of the sand and anthracite coal. Prior to filtration, the filter column was backwashed for about an hour to remove any silt and dust from the media. The settled water from the jar test procedure was pumped at 18.8 m/h from the gently stirred reservoir by using a peristaltic pump to the inlet of the filter head tank. A peristaltic pump was selected for this purpose to avoid breaking up the floc formed during coagulation and flocculation. A constant-head was maintained on the filter by virtue of the head-tank and overflow. Overflow flow rate was maintained at 12.8 m/h. Flow rate through the filter at the beginning of the filtration run was 4.9 m/h. The filtered water generated during the filtration run was collected as a composite sample. Sub-samples were also collected at various times during the filtration run, and the turbidity, absorbance and spore concentration were measured. Typical flow rates at different points on the filtration column are shown in Table 3-3.

Table 3-2 Physical properties of sand and anthracite coal (AWI Filter)

Property	Sand	Anthracite Coal
Bulk density	1362 kg/m^3 (dry basis)	N/A
Sphericity	< 0.6 (highly angular)	N/A
Moh hardness	7	N/A
Acid solubility	Nil	N/A
Specific gravity	2.60 – 2.65	1.4 – 1.6 ± 0.05
Effective size	0.35 mm	0.8 – 0.9 mm
Uniformity coefficient	< 1.5	N/A

N/A – Not Applicable

3.6 UV EXPOSURE

Samples of filtered and filter backwash water were exposed to controlled doses of UV in accordance with the collimated beam apparatus protocol suggested by Bolton and Linden (2003) but with some modifications. Table 3-3 provides a description of the samples used in this study. In Phase 1 experiments, two sub-samples of ~ 400 mL each were collected from the composite samples of filtered and filter backwash water. The first sub-sample was homogenized at 30 000 rpm for 3 min before being exposed to UV. The second sub-sample was exposed to UV first and then homogenized. Spores suspended in pH 8.0 phosphate buffered DI water were also exposed to UV radiation. The Phase 1 UV exposures were randomized between replicates of the same water matrix, but in the Phase 2 experiments, one of the three sub-samples of ~ 400 mL of filtered and backwash water was homogenized whereas the other two were not. After UV exposure, one of the two non-homogenized sub-samples was homogenized before enumeration whereas the other was not homogenized. In this phase, three experimental conditions were investigated: homogenized before UV exposure, homogenized after UV exposure, and non-homogenized. The practical implications of these experimental conditions are discussed in Chapter 5. As in Phase 1, spores suspended in pH 8.0 phosphate buffered DI water were also exposed to UV radiation, but in Phase 2, two water matrices having different spore concentrations were exposed to UV radiation. The different spore concentrations were used to measure the effect of concentration on the UV inactivation of *B. subtilis* spores. Spores were added to filtered water to investigate whether directly adding spores to water samples containing particles was different than adding spores to raw water and then taken through upstream treatment processes. Phase 2 UV exposures were completely randomized between replicates and water matrix.

Twenty millilters of the water samples (Table 3-4) were exposed to a pre-determined UV dose of 0, 10, 20 or 40 mJ/cm^2 in a 48 mm ID glass Petri dish. The suspensions were continuously but gently stirred during exposure by using of a 10 mm x 3 mm Teflon-coated stir bar. The irradiance at the center of the beam and at the surface of the liquid was measured by using a calibrated radiometer and detector (P-9710 Optometer and UV-3710 Irradiance Detector, Gigahertz Optik, Newburyport, MA). This measurement was converted into a depth-averaged irradiance by correcting for the radial variation of irradiance at the liquid surface, reflection at the air-water interface, absorbance and divergence within the water column. In Phase 1, the absorbance at 254 nm of each suspension was measured by using a conventional UV-Vis spectrophotometer (Ultrospec 2000, Pharmacia Biotech, Cambridge, England). However, in Phase 2 experiments, a UV-Vis spectrophotometer (UV-2401PC, Shimadzu Corp., Columbia, Maryland) equipped with an integrating sphere attachment (ISR 2200) was used. With the integrating sphere, both transmitted and forward and side scattered radiation were accounted for in the determination of the depth-averaged irradiance using the Beer-Lambert law. A comparison between the measurements taken with an integrating sphere attachment and a conventional spectrophotometer was conducted.

Table 3-3 Description of water matrices exposed to UV radiation.

Sample Number	Phase 1	Phase 2
1	Filtered water (homogenized after UV exposure)	Backwash water (non-homogenized)
2	Filtered water (homogenized before UV exposure)	Seeded phosphate buffered DI water (CFU = 10^5/mL)
3	Backwash water (homogenized after UV exposure)	Filtered water (homogenized before UV exposure)
4	Backwash water (homogenized before UV exposure)	Backwash water (homogenized before UV exposure)
5	Seeded phosphate buffered DI water (No pasteurization after UV)	Seeded filtered water
6	Seeded phosphate buffered DI water (Pasteurization at 80°C for 15 min	Seeded phosphate buffered Water (CFU = 10^7/mL)
7	ND	Filtered water (non-homogenized)
8	ND	Filtered water (homogenized after UV exposure)
9	ND	Backwash water (homogenized after UV exposure)

ND – Not Done

After exposure, ~ 10 mL sub-samples were removed from the Petri dish and transferred to sterile test tubes. For samples homogenized after UV exposure, the 10 mL sub-samples in the test tubes were homogenized at 30 000 rpm for 30 s. Samples were then enumerated for *B. subtilis* spores using the pour plate technique, as described previously. Sub-samples were pasteurized at 75°C for 15 min prior to enumeration because it was shown that post UV exposure pasteurization had little effect on the UV dose response for temperatures up to 80ºC (Section 4.1.4), which provided a safety margin of 5ºC. This procedure was performed to inactivate vegetative bacteria and to ensure that only spores were plated and enumerated. Inactivation was computed as – $\log_{10}$ (N/N_0), where N_i and N_f were the concentration of viable spores (in CFU/mL) in the control (zero dose) and the UV-exposed suspensions, respectively. All UV exposures were carried out in triplicate, and each exposed sample was enumerated in triplicate. The initial concentration, N_i of spores in the water samples (Table 3-4) was determined as an average of triplicate zero doses that were completely randomized with other samples.

The positive control samples for the UV exposure experiments were zero dose exposures, whereas negative controls were conducted for the dilution water, growth medium and indigenous spores in raw water. For the zero dose exposures, the collimated beam apparatus was turned on, but the shutter was closed while the samples were being stirred (power on shutter closed controls). The positive control samples for each water sample were treated exactly like the other samples. Samples were left in this mode for 3 min before enumeration. As a negative control, sterile phosphate buffered DI water was exposed to UV radiation. For other negative control samples, 1 mL of agar and dilution water were placed in Petri dishes and incubated at 37ºC for 48 h. The procedure for indigenous spore enumeration was described in Section 3.3.1. The effect of a post-UV exposure pasteurization step was also studied. *B. subtilis* spores suspended in phosphate buffered DI water were heated in a water bath (Fisher Scientific, Pittsburgh PA) set at 80ºC for 15 min. A comparison of the pasteurized and un-pasteurized samples was carried out.

3.7 MEASUREMENT OF PARTICLE-ASSOCIATION BY SERIAL FILTRATION

Studies have shown that the isolation and removal of particle-associated microorganisms have improved the inactivation rate constant (Liltved and Cripps 1999; Qualls et al. 1983). In this present study, however, a serial filtration was conducted to determine the size distribution of the particles in the filtered water. Also, the percentage of microorganisms associated with particles at specific cut-off sizes was determined. The replicate filters from the serial filtration step were examined under a scanning electron microscope to qualitatively assess particle-association.

A 150 mL portion of filtered water was placed in a clean 200 mL beaker that was washed with particle-free water. Triplicate samples were serially filtered through 10, 5, 2, and 0.4 μm polycarbonate track etched membrane filter (Isopore™ membrane filters, Millipore, Ireland) by using a membrane filtration glassware with fritted glass support (Kontes, Vineland, NJ) and a vacuum filtration apparatus. The vacuum pressure on the pump was maintained at 14 kPa. The particles retained on the membrane filter were washed off with a sterile rubber policeman into 150 mL sterile pH 8.0 phosphate buffered DI water. The suspension was homogenized for 3 min at 30 000 rpm and enumerated. Ten milliliers sub-samples were pasteurized at 75°C for 15 min before enumeration. Non-homogenized and homogenized samples of the filtered water were also enumerated. The number of particles passing through the PCTE filter was determined by using a Hiac-Royco Particle Counting System. The cumulative and differential particle counts for ≥ 2, ≥ 5, ≥ 7, ≥10, ≥ 14, ≥ 20, ≥ 40, and ≥ 80 μm were reported by the particle counter.

3.8 ELECTRON MICROSCOPY

After filtration, the replicate membrane filters were fixed with 2% glutaraldehyde and placed in sterile 150 x 15 mm polystyrene Petri dishes. Square samples of about 15 mm x 15 mm were cut from the center of the membrane filter. The samples were gently rinsed in a series of 20, 40, 60, 80 and 100% ethanol for 15 min each. The samples were further rinsed through another series of 20/80, 40/60, 80/20% v/v combination of ethanol and hexamethyldisilazane (HMDS) for 15 min each. Two 15-min washes in 100% HMDS were done before air-drying the samples on a filter paper. All washing involving HMDS was carried out in a fumehood. The dried filter samples were attached to a metal stub with glue. Stubs containing membrane filters were sputtered with a thin layer of gold before being mounted on a Scanning Electron Microscope (SEM) (Philips LaB_6 ESEM Electron Scanning Microscope, USA). Membrane filters were examined at an accelerating voltage of 16 kV and various magnifications.

4 ANALYSIS OF RESULTS

This chapter presents the experimental results obtained in this study. The chapter is subdivided into five parts: water treatment simulation, control experiments, impact of upstream treatment processes on UV inactivation of *B. subtilis* spores, direct measurement of aggregation, and microscopic examination of aggregation.

4.1 CONTROL EXPERIMENTS

4.1.1 Negative Controls

Experimental controls were necessary to ensure that unknown variables were not introduced into experimental results. The absence of growth on the negative controls suggests that no contamination of enumerated plates occurred. Hence, *B. subtilis* spores were not introduced through the dilution solution and growth medium and that the aseptic procedures were satisfactory.

4.1.2 Impact of Indigenous Spores on Microbial Counts

Table A-1 in Appendix A presents indigenous spore enumeration data for Phases 1 and 2. The average spore concentration in the river water was about 7 CFU/mL for Phases 1 and 2. Therefore, the ratio of indigenous aerobic spores to cultured *B. subtilis* spores in the seeded raw water was about 1 to 1 000 000. The low numbers of indigenous spores present in the raw water were expected to have little impact on the experimental results.

4.1.3 Batch-to-Batch Variation of Spore UV Inactivation Characteristics

Because the experiments in Phases 1 and 2 were conducted several months apart, the possible batch-to-batch variation of the response of *B. subtilis* spores to UV radiation had to be understood. Three experimental trials from two stock suspensions were conducted to determine this variation. Trial 1, conducted in Phase 1 (November 2004), was from the first stock suspension whereas Trials 2 and 3, conducted in Phase 2 (August 2005), were from the second stock suspension. However, different densities of spores were used in

Trial 2 and Trial 3 experiments. Spore concentrations were 1.6 x 10^7 CFU/mL and 1.4 x 10^6 CFU/mL in Trial 2 and Trial 3, respectively. Figure 4-1 shows the batch-to-batch variation of the UV inactivation of *B. subtilis* spores suspended in phosphate buffered DI water at 22°C. Analysis of variance (ANOVA) indicated that there was a statistical difference between the inactivation curves at a 95% confidence level (*p*-value = 3.5 x 10^{-11}, n = 36).

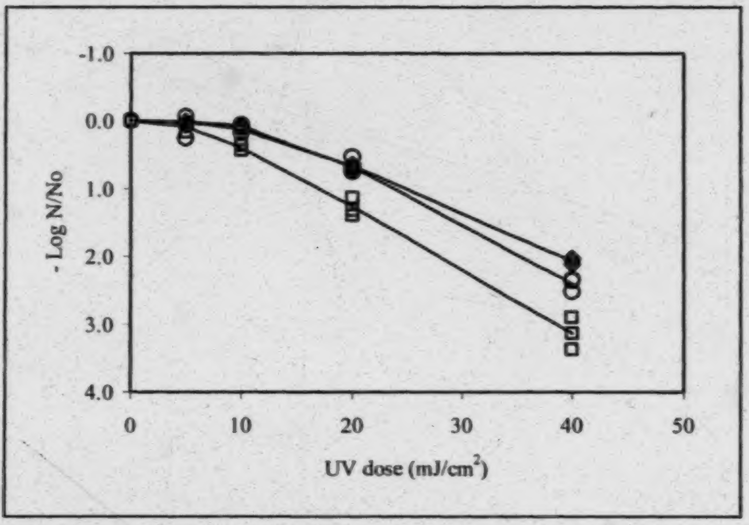

Figure 4-1 Batch variation of the UV inactivation of seeded *B. subtilis* spores in phosphate buffered DI water at 22°C used in Trial 1 (□), Trial 2 (*) and Trial 3 (◇) representing Phases 1 and 2.

Because the null hypothesis was rejected, the Scheffe's least square difference (LSD) test was used to determine at which doses the differences were significant. This test was selected instead of the Fisher's LSD test because the Scheffe's test was more likely than the Fisher's LSD test to reject the null hypothesis. The details of the Scheffe's LSD test are presented in Table A-12 Appendix A. No significant differences were found between all pairs of mean UV inactivation for Trials 2 and 3 except at 40 mJ/cm^2. This difference may have been due to the bias introduced by serial dilution that shielding may not account for. However, significant differences were found between all pairs of mean UV inactivation for Trial 1 and Trials 2 and 3 except at 5 mJ/cm^2. This difference between the UV inactivation in Trial 1 and Trials 2 and 3 was expected because Trial 1 was from a different stock suspension than Trials 2 and 3.

A multi-target model (Equation 2-12) was fitted to the UV inactivation data (Tables B-6, C-2 and C-6 in Appendix B and C). The Microsoft® Excel® Solver tool was used to minimize the sum of squares of the predicted and experimental values to obtain model parameters. The multi-target model parameters, number of targets, n_c, were 4, 14 and 7 while the rate constants, k_m, were 0.22, 0.20 and 0.17 cm^2/mJ for Trials 1, 2 and 3, respectively. The predictions of inactivation for Trials 2 and 3 were similar at all doses except 40 mJ/cm^2 (Figure 4-1). Because the inactivation rate constants for Trial 1 (k_m = 0.20) and Trial 2 (k_m = 0.22) were close, Trial 2 inactivation data were selected for the comparisons with the filtered and backwash water trials conducted in Phase 2 of this study. Model results were consistent with those from the Scheffe's LSD test.

4.1.4 Effect of Post Pasteurization on UV Inactivation

Because *B. subtilis* spores were suspended in river water so that the effects of upstream treatment processes on UV inactivation could be measured, contamination by vegetative bacteria present in the water had to be minimized. This procedure was done by heating samples to 80°C after they had been exposed to UV radiation and prior to enumeration. In order to determine the effect of this pasteurization step on the measured UV inactivation of the spores, an experiment was conducted in which spores in sterile DI water were exposed to several UV doses, with and without pasteurization following UV exposure. The results are presented in Figure 4-2. The UV inactivation data of this test are presented in Table B-6 and B-7 in Appendix B.

A two-factor ANOVA test indicated that there was no statistical difference between the two treatment conditions at a 95% confidence level (p-value = 0.31, n = 24). When a multi-target model was fitted to the data, the n_c and k_m values were found to be 4 and 0.22 cm^2/mJ and 4 and 0.20 cm^2/mJ for the non-pasteurized and 80°C for 15 min pasteurization treatment conditions, respectively. The test results indicated that the pasteurization step had little effect on the measured UV inactivation of the spores. It was important to ensure that the pasteurization steps did not inactivate the spores or alter their resistance to UV radiation.

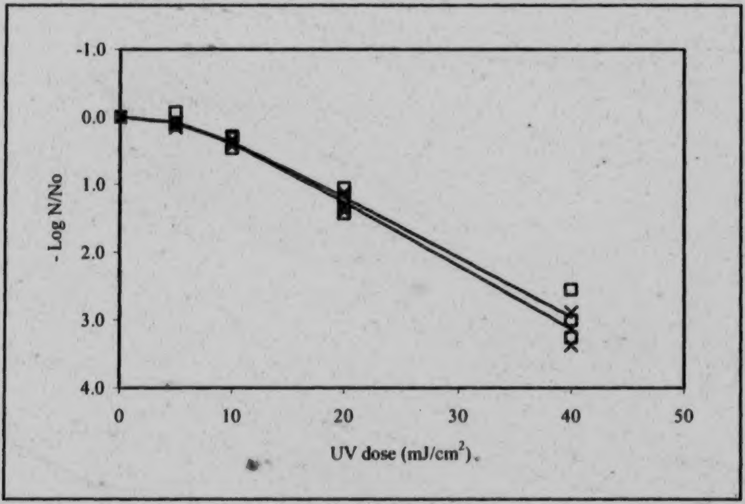

Figure 4-2 Impact of pasteurization on the UV inactivation of *B. subtilis*. Samples were either pasteurized at 80°C for 15 min (☐) before enumeration or not pasteurized (×).

4.1.5 Impact of Homogenization on Aggregation and Viability of *B. subtilis* Spores

The objective of these control experiments was to determine whether the homogenization had a significant impact on *B. subtilis* spore aggregation and viability. Three sets of experiments were carried out as follows. In the first set of experiments, two suspensions were prepared. The suspensions were enumerated each day for 3 days. The first suspension was homogenized on day 1 only, whereas the second suspension was homogenized each day over 3 d. The results, presented in Figure 4-3, indicated that the measured CFU of spores in the daily homogenized suspension was constant, while that in the suspension homogenized only on the first day decreased. An ANOVA test indicated that there was no statistical difference between the samples that were homogenized once and those that were homogenized daily (p-value = 0.56, n = 24). This result suggests that repeated homogenization did not reduce spore viability.

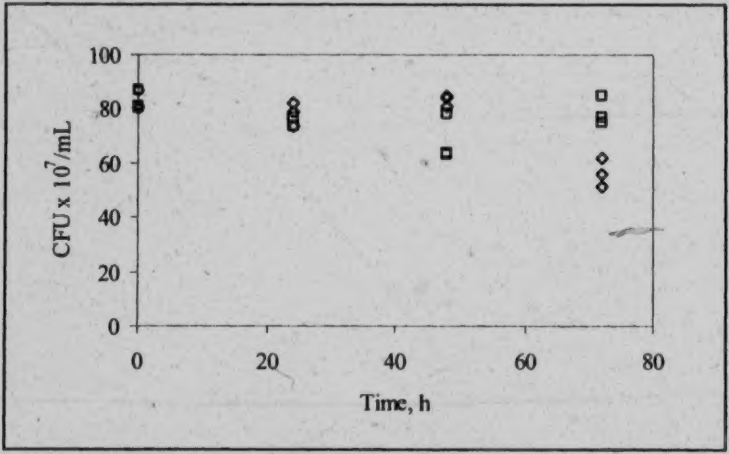

Figure 4-3 Impact of homogenization on the viability of *B. subtilis* spores. Spore suspensions were either homogenized once (◇) or daily (□) prior to enumeration.

For the second set of experiments, two sub-samples were taken daily from one spore suspension that had not been homogenized. One of the sub-samples was homogenized while the other was non-homogenized. Figure 4-4 shows the results of the second set of experiments. ANOVA indicated that there was a significant difference in the CFU between the homogenized and non-homogenized samples (p-value = 0.0054, n = 24). This finding suggests that the homogenized samples were consistently different from the non-homogenized samples for up to 3 d. The third set of experiments was similar to the second one except that the spore suspension was homogenized on day 1 only. Two sub-samples that were non-homogenized and homogenized were then taken and enumerated. The results in Figure 4-5 indicated that the CFU was increased after the initial homogenization and that the concentration was constant over time. ANOVA indicated that there was no statistical difference in spore concentration between homogenized and non-homogenized samples if the samples were initially homogenized (p-value = 0.71, n = 24).

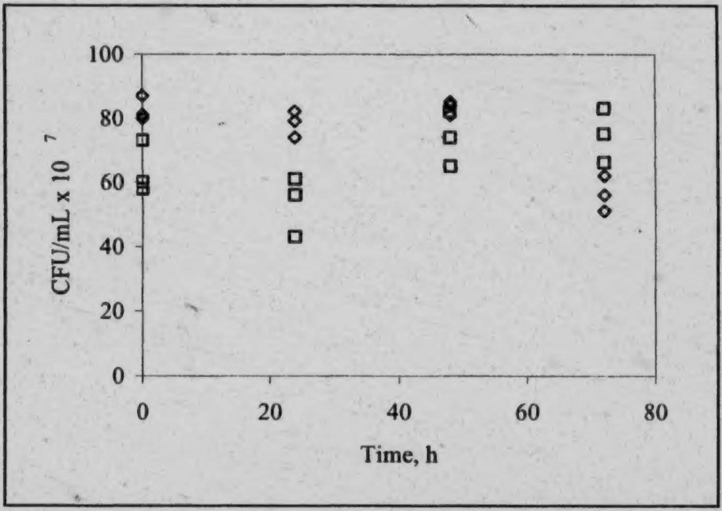

Figure 4-4 Impact of the homogenization on the viability of *B. subtilis* spores. Spore suspension was *not homogenized* on the first day, and then sub-samples were either homogenized (◇) or non-homogenized (☐) before enumeration on each subsequent day.

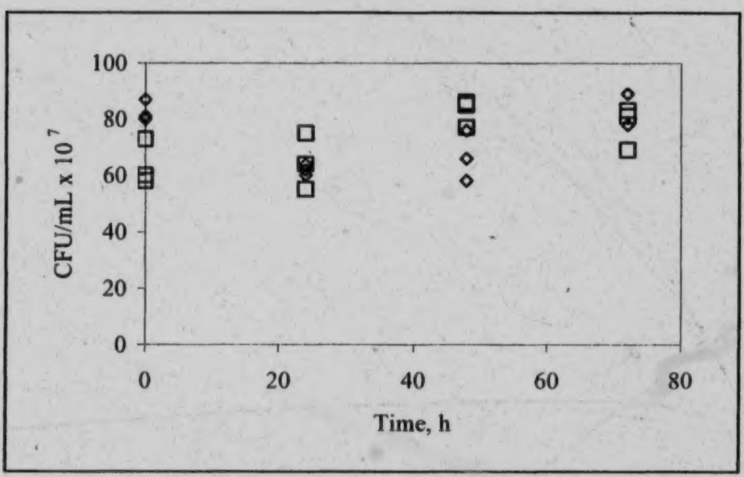

Figure 4-5 Impact of the homogenization on the viability of *B. subtilis* spores. Spores suspension was *homogenized* on the first day, and then 10 mL sub-samples were either homogenized (◇) or non-homogenized (☐) before enumeration on each subsequent day.

The impact of the homogenization step on the aggregation, viability and recovery of spores was investigated to determine the possible effects. Since there was no increase in the enumerated CFU, the speed and time setting selected for the homogenization step was sufficient to disperse the spores. Homogenization had no detectable effect on the viability of the spores. Figure 4-3 reveals an apparent drop in the measured CFU at 3 d, but this difference was not statistically significant. This result supports the conclusion that the homogenization step broke up aggregated microorganisms in the test suspensions and that little re-aggregation of spores occurred following homogenization for up to 3 d. Figures 4-3, 4-4 and 4-5 suggest that repeated homogenization did not damage the spores or reduce viability – the counts were essentially constant. In addition, homogenization did not have a statistically significant effect on spore concentration.

4.2 WATER TREATMENT SIMULATION

In this study, *B. subtilis* spores were seeded directly into the raw river water and then exposed to simulated coagulation, flocculation, settling and filtration conditions. Two filtration runs were done during different time periods of the study using different raw water. The filtration runs were identified as "Phase 1" and "Phase 2." For each filtration run, filtered and backwash water were collected, and a series of UV exposure experiments were done. In Phase 2, the UV exposure experiments were complemented with measurements of aggregation using the PCTE membrane filters and SEM. Because an integrating sphere spectrophotometer became available prior to Phase 2, forward and side scattering of UV radiation was accounted for in the transmission measurements made in Phase 2. Filtered and backwash water were collected as a composite sample. In Phase 1, filtered water was collected in a large container and mixed prior to use in experiments. See Table 4-1 for the absorbance of the water matrices exposed to UV radiation. However, in Phase 2, the composite sample was split into two. Water from the first 60 min of the filtration run was collected as Composite 1 whereas 60 to 180 min of the filtration run was collected as Composite 2 (Table 4-2).

4.2.1 Phase 1 Results

Figure 4-6 shows the turbidity of the filtered water as function of time for the Phase 1 filtration run. Settled water turbidity was 8.5 NTU. After an initial ripening period of approximately 60 min, the turbidity in the filtered water remained approximately constant at 1 NTU for the duration of the 360 min filter run. The filtered water turbidity of approximately 1 NTU was considerably greater than the USEPA's LT2ESWTR filtration standard of 0.3 NTU (USEPA 2003a) but comparable to the Health Canada maximum acceptable concentration of 1 NTU (Health Canada 2004). One of the objectives of this study was to determine the effects of aggregated microorganisms on UV inactivation during periods of sub-optimal coagulation. Therefore, the alum dose was intentionally adjusted to a sub-optimal condition after initial jar testing. Following the 360 min filtration run, the filter was backwashed, and the backwash water was collected.

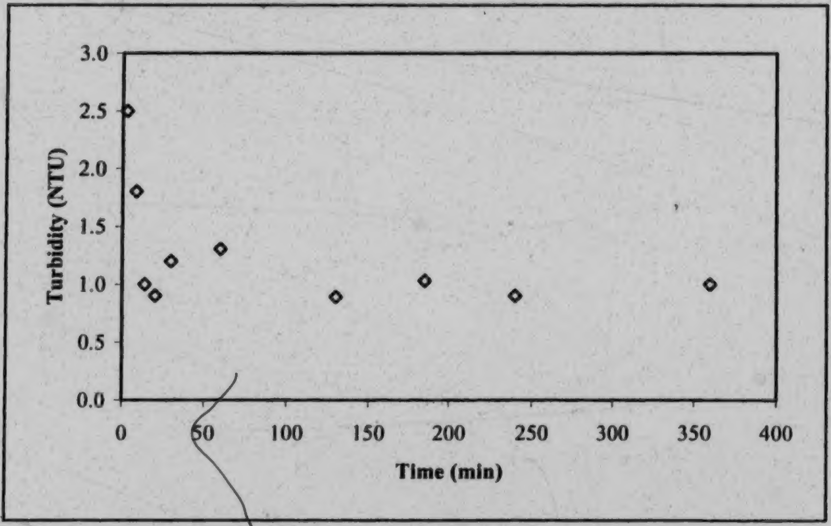

Figure 4-6 Filtered water turbidity as function of time for the Phase 1 filtration run.

The CFU counts in the filtered and backwash water were $2 \pm 0.6 \times 10^4$ /mL and $1.4 \pm 0.1 \times 10^6$ /mL respectively, which imply that there was a 2 log reduction in the viable spore concentration over the initial concentration ($2 \pm 0.4 \times 10^6$ spores/mL) added to the coagulation jars. These measurements are based on the mean ± 2 × std. error of the CFU determination in three replicate samples of the filtered and backwash water. Spore concentration in the filtered water was sufficient to permit measurement of up to 3 log inactivation in UV exposure experiments. Filtered and backwash water samples were homogenized either before or after UV exposure (Table 4-1). Spores suspended in pH 7 phosphate buffered DI water were also exposed to the same range of UV doses. UV doses of 0, 5, 10, 20 and 40 mJ/cm^2 were used, and each exposure was carried out in triplicate. The absorbance of the water matrices used in this phase is provided in Table 4-1.

Table 4-1 Absorbance of water matrices used in Phase 1 experiments. Filtrate was collected as a composite sample.

Experimental condition	UV absorbance[a] (cm^{-1})
Filtered water (homogenized before UV exposure)	0.055
Filtered water (homogenized after UV exposure)	0.057
Backwash water (homogenized before UV exposure)	0.110
Backwash water (homogenized after UV exposure)	0.058
Spores suspended in pH 7 phosphate buffered DI water[b]	0.070

[a] Absorbance was measured using a conventional UV-Vis spectrophotometer at 254 nm.

[b] Spore stock suspensions were homogenized before they were added to DI water.

4.2.2 Phase 2 Results

The variation of filtered water turbidity and spore concentration with time during the Phase 2 filtration run is provided in Figure 4-7. Additional treatment information pertaining to this filtration run is provided in Table 4-2. Filtered water turbidity (composite) was approximately 1 NTU. After an initial ripening period of approximately

60 min, the filtered water turbidity stabilized at approximately 0.6 to 0.7, NTU which was again considerably greater than the USEPA's LT2ESWTR filtration standard of 0.3 NTU but less than the Health Canada guideline of 1 NTU. The spore concentration in the raw river water was reduced by approximately 3 log units by the simulated conventional treatment. Nevertheless, viable spores were present in the filtered effluent at a concentration of approximately 1 x 10^4 CFU/mL. This concentration was sufficient to carry out UV inactivation trials using a collimated beam apparatus with 2 to 3 log inactivation sensitivity.

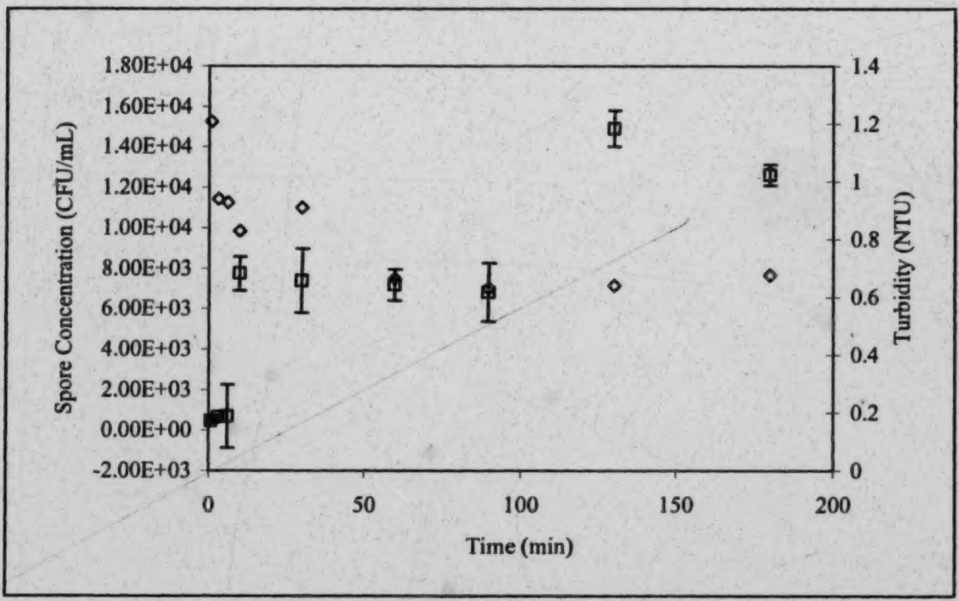

Figure 4-7 Turbidity (◇) and *B. subtilis* spores concentration (□) in filtered water as a function of time after the start of the Phase 2 filtration run. Error bars represent 2 x std. error of triplicate plate counts.

Table 4-2 Reduction of turbidity, spore concentration and UV absorbance before and after simulated water treatment in Phase 2.

Water sample	Turbidity (NTU)	*B. subtilis* spore conc. (CFU/mL)	UV Absorbance	
			Conventional transmission measurements[a]	Integrating sphere measurements[b]
Raw (after seeding)	64.2	$5.7 \pm 2.5 \times 10^7$	0.514	0.375
Settled	13.8	$1.7 \pm 1.8 \times 10^5$	0.070	0.071
Filtered – Composite 1	0.7	$1.0 \pm 0.04 \times 10^4$	0.061	0.059
Filtered – Composite 2	0.7	$8.9 \pm 2.9 \times 10^3$	0.061	0.059
Backwash Water	5.8	$1.0 \pm 0.3 \times 10^6$	0.022	0.018

[a] Based on conventional UV transmission measurements in a 10 mm quartz cuvette.

[b] Based on UV transmission and forward and side scattering measurements made in an ISR2000 integrating sphere with a 10 mm quartz cuvette.

4.3 IMPACT OF TREATMENT PROCESSES ON UV INACTIVATION

In order to measure the number of viable spores in the filtered and backwash water samples – including those that may have been aggregated – samples were homogenized before and after exposure to UV doses of 0, 5, 10, 20 and 40 mJ/cm^2. UV exposures were done in triplicate. Split samples were also homogenized prior to UV exposure so that aggregated spores were now exposed to UV radiation. In Phase 2, a third set of UV exposures was conducted in which the spore suspensions were homogenized either before or after UV exposure. In samples that were homogenized before UV exposure, spores were more dispersed in the suspensions, and hence, the spores were more likely to be exposed to the same UV dose. However, in samples that were homogenized after UV exposure, and non-homogenized samples, spores that may have been aggregated or engulfed in floc may not have received the same UV dose as dispersed spores. The results obtained from samples that were homogenized before or after UV exposure and samples that were non-homogenized are presented in this section. This section is further sub-divided into Phase 1 and Phase 2 results.

4.3.1 Phase 1 Results

4.3.1.1 Backwash water

The UV inactivation curves of *B. subtilis* spores present in the backwash water from the Phase 1 filtration run are presented in Figure 4-8. Raw data for Phase 1 UV exposure results are provided in Tables B-4, B-5 and B-6 in Appendix B. Three inactivation curves are shown in each figure:

(1) An inactivation curve generated using water samples that were homogenized after UV exposure;

(2) An inactivation curve generated using water samples that were homogenized before UV exposure, and

(3) An inactivation curve generated using clean spore preparations that were dispersed directly into buffered DI water (note: spore stock suspensions were homogenized before they were added to the buffered DI water).

Each inactivation curve is characterized by a shoulder region at a low UV dose and a region of approximately first-order, linear inactivation at a higher dose. The effect of aggregation on the measured UV inactivation characteristics of the spores is apparent by comparing spore inactivation for suspensions that were homogenized before UV exposure to spore inactivation for suspensions that homogenized after UV exposure. In Figure 4-8, for example, the inactivation of spores in samples that were homogenized before UV exposure is noticeably greater than inactivation of spores in samples that were homogenized after UV exposure. At 40 mJ/cm^2, the inactivation of spores in backwash water samples that were homogenized before UV exposure ranged from 2.4 to 2.5 log units whereas samples that were homogenized after UV exposure was in the range of 0.6 to 0.9 log units. ANOVA test indicated that there was a statistical difference between the inactivation of spores in backwash water samples that was homogenized before UV exposure and the inactivation of spores in backwash water samples that were homogenized after UV exposure (p-value = 4.8 x 10^{-11}, n = 24). Table 4-3 shows the parameters, number of targets (n_c) and inactivation rate constant (k_m) of the multi-target model used to fit the inactivation data. The UV inactivation rate constant, k_m of spores in the backwash water increased 3.6 times, from 0.05 to 0.18 cm^2/mJ, when the homogenization step was carried out before UV exposure versus after UV exposure (Table 4-3). The inactivation rate constant, k_m of clean spores dispersed into buffered DI water was 0.22 cm^2/mJ, which is comparable to the inactivation rate constant of spores in backwash water that was homogenized prior to UV exposure (0.18 cm^2/mJ). The proposed explanation for this observation is that some of the spores present in the backwash water were aggregated and embedded within particulate matter and were partially protected from UV radiation during the exposure. The homogenization procedure broke the aggregates apart. The accompanying release of embedded spores into the water as mono-dispersed spores resulted in an apparent increase in the susceptibility of the spore population to UV radiation.

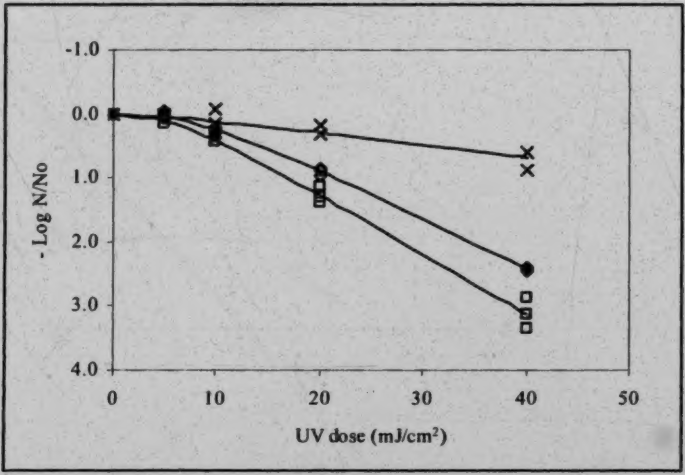

Figure 4-8 UV inactivation of *B. subtilis* spores present in filter backwash water. Samples were homogenized before (◇) and after (×) UV exposure. Inactivation curve of clean preparations of *B. subtilis* spores suspended in buffered DI water is also shown (□).

Table 4-3 Least square model parameters and rate constants for multi-target model fit to the inactivation data of spores in backwash and filtered water (Phase 1)

Experimental conditions	Backwash water		Filtered water	
	Number of targets, n_c	Rate constants, k_m	Number of targets, n_c	Rate constants, k_m
Homogenized before UV exposure	5	0.18	1	0.21
Homogenized after UV exposure	2	0.05	1	0.16
Phosphate buffered DI water[a]	4	0.22	4	0.22

[a] The same parameters appear under backwash and filter water columns because only one set of UV exposure trials was conducted on phosphate buffered DI water.

4.3.1.2 Filtered water

A similar but less distinctive observation was made during the filtered water experiments (Figure 4-9). At 40 mJ/cm^2, the inactivation of spores in samples that were homogenized before UV exposure ranged from 3.6 to 3.9 log units whereas the inactivation of spores that were homogenized after UV exposure ranged from 2.4 to 3.1 log units. The inactivation of spores that were dispersed in phosphate buffered DI water was in the range of 2.9 to 3.4 log units at 40 mJ/cm^2. ANOVA test results indicated that there was a statistical difference between the inactivation of filtered water samples that were homogenized before UV exposure and the inactivation of samples that were homogenized after UV exposure (p-value = 2.7×10^{-7}, n = 24).

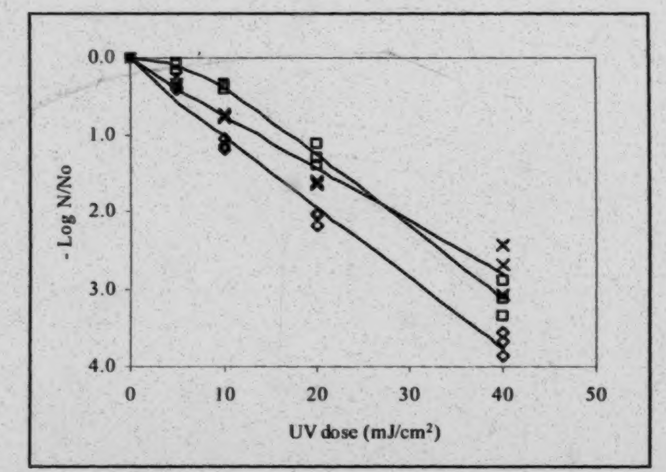

Figure 4-9 UV inactivation of *B. subtilis* spores present in filter effluent; Samples were homogenized before (◇) and after (×) UV exposure. Inactivation curve of clean preparations of *B. subtilis* spores suspended in buffered DI water is also shown (☐).

Parameters of the multi-target model fit to the inactivation data of spores in filtered water can be found in Table 4-3. The inactivation rate constant, k_m, of spores in the filtered water was increased 1.3 times, from 0.16 to 0.21 cm^2/mJ, when the homogenization step was carried out before UV exposure versus after UV exposure. In the filtered and backwash water UV exposures, the inactivation rate constant of spores in samples that were homogenized before UV exposure was greater than the inactivation rate constant of spores in samples that were homogenized after UV exposure but close to the inactivation rate constant of spores in clean buffered DI water. This finding suggests that the degree of aggregation in the filtered water was much lower than in the backwash water. However, some fraction of the spores in the filtered water was embedded within the particles that had broken through the filter, and thus, had been protected from UV exposure. The number of targets, n_c was different for the spores in the backwash and filtered water (Table 4-3). The reason for the difference was not obvious but it could be that enmeshment and aggregation may have contributed to this difference through the reduction of the possible targets on the spores. The n_c values for samples that were homogenized before UV exposure were consistently higher than those that were homogenized after UV exposure. Another possible reason is that a modeling artifact was created when the least squares values were fitted to the inactivation data because k_m and n_c are highly correlated.

The inactivation of the spores in the filtered water samples that were homogenized prior to UV exposure exceeded the inactivation of clean spores dispersed in buffered DI water. The difference, however, was not statistically significant. This unusual result may have resulted from the incomplete randomization of filtered and backwash water samples in Phase 1. As a result, a least square difference test was not conducted to determine which differences were significant. In Phase 2, however, a complete randomization of all samples was implemented.

4.3.2 Phase 2 results

4.3.2.1 Backwash water

Results of the UV inactivation of *B. subtilis* spores present in backwash water collected during the backwash cycle of the Phase 2 filtration run are presented in Figures 4-10 and 4-11. The experimental data for Figures 4-10 and 4-11 are provided in Tables C-1, C-4, C-6 and C-9 Appendix D. At the low UV doses (20 mJ/cm^2 or less), inactivation was limited to less than 1 log and was independent of the method of exposure. At the highest UV dose tested (40 mJ/cm^2), on the other hand, the level of inactivation was determined by whether or not the homogenization step was carried out before or after UV exposure. In samples that were homogenized prior to UV exposure, inactivation at 40 mJ/cm^2 was approximately 1.3 to 2.0 log (Figure 4-10). For those samples that were homogenized after UV exposure, inactivation was limited to approximately 0.3 to 0.6 log units. In comparison, inactivation of prepared spores suspended in particle-free phosphate buffered DI water was approximately 2.3 to 2.5 log units at the same dose. As in Phase 1, prepared spores were homogenized before they were added to the DI water samples. This was taken as evidence that a fraction of the spore population in the backwash water was aggregated with particles and was thus protected from UV exposure.

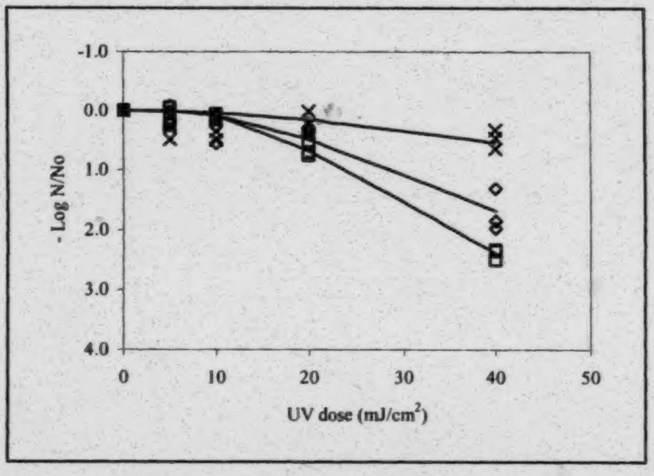

Figure 4-10 UV inactivation of *B. subtilis* spores in filter backwash water that was homogenized before (◇) and after (×) UV exposure compared to spores that were seeded (□) into pH 8 phosphate buffered DI water.

A comparison of spore inactivation in backwash water samples that were homogenized before and after UV exposure and samples that were not homogenized (either before or after) is provided in Figure 4-11. Inactivation of spores in non-homogenized samples was approximately 0.3 to 1.3 log units at 40 mJ/cm^2. In addition, it was observed that inactivation of spores in the non-homogenized samples was greater than the inactivation of spores in samples that were homogenized after UV exposure but was less than spore inactivation in samples that were homogenized before UV inactivation. This was another indication that spores were aggregated in backwash water samples. In addition, the comparison of the inactivation of spores in samples that were homogenized before and after UV exposure to the inactivation of spores in non-homogenized samples emphasizes the importance of the homogenization step. The average CFU of non-homogenized samples was 5.2 x 10^5 CFU/mL whereas samples that were homogenized before UV exposure had 7.8 x 10^5 spores/mL. The viable spore concentration of samples that were homogenized before UV exposure was 1.5 times greater than the viable spore concentration in non-homogenized samples. This difference in viable spore concentration provides a direct indication of the level of aggregation even though the aggregate size and distribution were unknown.

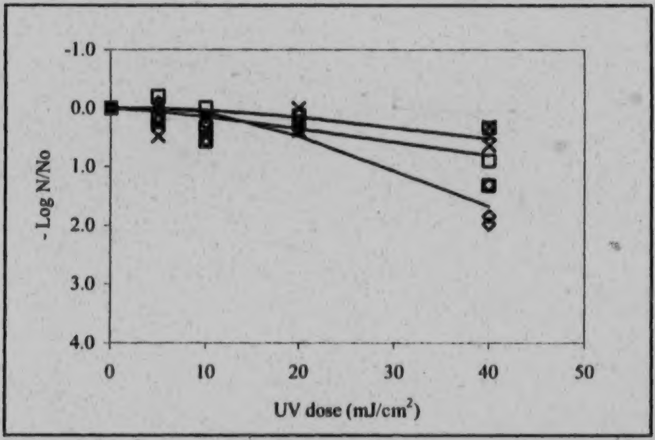

Figure 4-11 Comparison of UV inactivation of *B. subtilis* in backwash water samples that were homogenized before (◇) and after (×) UV exposure to non homogenized samples (□).

An ANOVA test on all four treatments – homogenized before and after UV exposure, non-homogenized and spores in buffered DI water indicated that there was a statistical difference in the treatments (p-value = 9.2 x 10^{-6}, n = 48). Because the null hypothesis was rejected, Scheffe's LSD test was conducted to determine which treatments were significantly different. The test results showed that all treatments were significantly different at 40 mJ/cm^2 except for the pair of inactivation of spores in non-homogenized and homogenized after UV exposure samples. This may have been as a result of the high number of aggregated and particle-associated spores in the backwash water. All pairs of treatment were found to be significantly different at 20 and 40 mJ/cm^2. This provides further evidence that a fraction of the spores in backwash water were either aggregated with other spores, with particles, or both.

The multi-target model parameters, number of targets, n_c and rate constants, k_m, for the inactivation of spores in backwash water that were computed using least-squares are provided in Table 4-4. The k_m for samples that were homogenized before UV exposure and non-homogenized samples were almost identical. But the rate constant, k_m of samples that were homogenized before UV exposure was 2.7 times greater than the rate constant of samples that were homogenized after UV exposure. This result gives an indication of rate of inactivation underestimation that may occur if aggregation and particle-association are not accounted for – that is, if homogenization is not used.

Table 4-4 Multi-target model fit to Phase 2 inactivation data of *B. subtilis* spores in backwash water.

Experimental conditions	Number of targets, n_c	Rate constants, k_m (cm^2/mJ)
Homogenized before UV exposure	7	0.15
Homogenized after UV exposure	3	0.055
Non-homogenized	1	0.056
Suspended in phosphate buffered DI water	14	0.20

4.3.2.2 Filtered Water

The results of UV exposure trials carried out with the filter effluent are presented in Figures 4-12 and 4-13 and the raw data are provided in Table C-3, C-6, C-7, and C-8 Appendix C. As with backwash water, the level of inactivation was influenced by the homogenization step but the effects were evident at only the highest UV dose of 40 mJ/cm^2. Inactivation of samples that were homogenized prior to UV exposure (2.1 to 2.5 log units) was essentially identical to inactivation of prepared spores suspended in particle-free phosphate buffered DI water (2.3 to 2.5 log units) at the 40 mJ/cm^2 dose (Figure 4-12). Also, inactivation in the samples that were homogenized after UV exposure (0.4 to 1.0 log units) was lower relative to that of samples that were homogenized prior to UV exposure (2.1 to 2.5 log units) at the 40 mJ/cm^2 dose. This is evidence that, even in the filtered water, a fraction of the spore population was likely aggregated with particles, and that spores may have been protected from UV exposure.

Figure 4-13 comparers the UV inactivation of samples that were homogenized before and after UV exposure and non-homogenized samples. The inactivation of non-homogenized samples was in the range of 2.1 to 2.2 log units. The difference in the UV inactivation in between the samples that were homogenized before UV exposure and the non-homogenized samples was small but consistent. A similar observation was made for the backwash water samples (Figure 4-11). This finding suggests that spores were aggregated in both filtered and backwash water, but to a lesser extent in the latter.

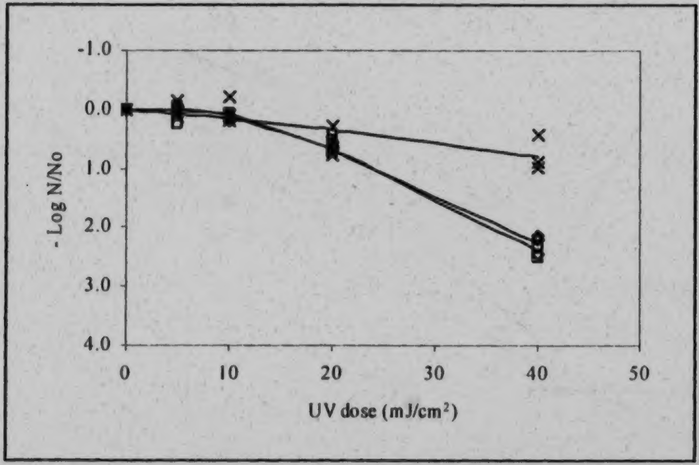

Figure 4-12 UV inactivation of spores present in filter effluent that was homogenized before (◇) and after (×) UV exposure compared to spores suspended in pH 8 phosphate buffered DI water (□).

A two-factor ANOVA test was conducted on all 4 treatments – homogenized before and after UV exposure, non-homogenized filtered water and buffered DI water samples – indicated that there was a statistical difference (p-value = 1.9×10^{-9}, n = 48). Hence, the null hypothesis was rejected. Scheffe's LSD test was then used to determine which pairs of means were significantly different. Again, the test indicated that there was a significant difference at 40 mJ/cm^2 between the samples that were homogenized after UV exposure and the other three treatments (homogenized before UV exposure, non-homogenized, and buffered water). These results show that the spores present in filtered water were aggregated with other microorganisms and to other materials present. The average CFU count in non-homogenized filtered water was 7.2 x 10^3/mL whereas filtered water samples that were homogenized before UV exposure had an average spore concentration of 1.4 x 10^4/mL. The CFU count of samples that were homogenized before UV exposure was 1.9 times greater than the spore concentration in non-homogenized samples. Like in backwash water samples, this difference directly indicates that a fraction of the spores in the filtered water were also aggregated.

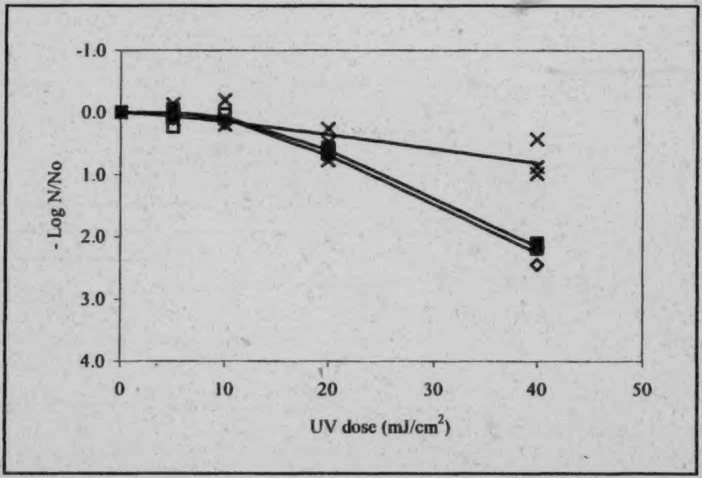

Figure 4-13 Comparison of UV inactivation of *B. subtilis* in filtered water samples that was homogenized before (◇) and after (×) UV exposure to non-homogenized samples (□).

Multi-target model parameters determined by least square for the inactivation of spores in filtered water are provided in Table 4-6. The inactivation rate constant, k_m of spores in filtered water samples that was homogenized after UV exposure was 3.3 times less than the inactivation rate constant of spores in non-homogenized samples. The inactivation rate constant of spores in filtered water samples that were homogenized before was 3.5

times greater than the inactivation rate constant that was homogenized after UV exposure. This finding implies that the true rate constant of *B. subtilis* spores in filtered water will be underestimated if aggregated and particle-associated spores are not broken up or accounted for. The rate constants of spores in filtered water samples that were homogenized before UV exposure and those of spores in phosphate buffered DI water were almost identical. This finding shows that samples that were homogenized before UV exposure contained aggregated spores that were disaggregated before exposure to UV. Like in Phase 1, the n_c spores in the backwash water were different than the those of the filtered water.

Table 4-5 Multi-target parameters, number of targets, n_c and rate constant, k_m, of multi-target model fitted to Phase 2 inactivation data of *B. subtilis* spores in filtered water.

Experimental conditions	Number of targets, n_c	Rate constants, k_m (cm^2/mJ)
Homogenized before UV exposure	9	0.19
Homogenized after UV exposure	1	0.055
Non-homogenized	11	0.18
Phosphate buffered DI water	14	0.20

At this point, it is necessary to distinguish between the true and apparent rate constants obtained from the kinetic modeling of the inactivation of *B. subtilis*. The multi-target model assumes that every cell has n_c targets. Therefore, it is reasonable to assume that the probability of a UV photon hitting a target is equal. Rate constants that satisfy both assumptions will be ideal and can therefore be described as *true* inactivation rate constants. In relation to this study, the inactivation of spores that were dispersed in filtered, backwash, and phosphate buffered DI water prior to UV exposure will most likely satisfy the assumptions of the multi-target model because the targets on the dispersed spores will have equal probabilities of being hit by UV photons. However, for aggregated or engulfed spores, the probability of a target being hit by a UV photon will be less than the probability of a target being by a UV photon on a dispersed spore. Shielding becomes a limiting condition for the inactivation of spores in aggregated and engulfed spores. Hence, in samples that were either non-homogenized or homogenized after UV exposure, the rate constant could be described as an *apparent* rate constant. The apparent rate constant of non-homogenized samples will be different than that of samples that were homogenized after UV exposure. This difference gives two levels of apparent rate constants because the homogenization step released aggregated spores that contributed to the higher CFU counts in the samples that were homogenized after UV exposure.

4.3.3 Comparison of the Inactivation of Spores in Filtered Water and Inactivation of Spores Added to Filtered Water Containing Particles.

In Figure 4-14, the inactivation of spores present in the filtered water but originally added directly to raw water is compared to inactivation of spores that were added directly to filtered water containing particles. Data for Figure 4-14 can be found in Tables C-3, C-5, C-6 and C-8 Appendix C. This comparison illustrates that adding microorganisms directly into filtered water samples containing particulate matter is not an appropriate method for predicting microorganism inactivation by UV in filtered water. Addition of indicator or surrogate microorganisms into raw water upstream of the coagulation process produces a more realistic assessment of UV inactivation in filtered water because this technique accounts for the true aggregation potential.

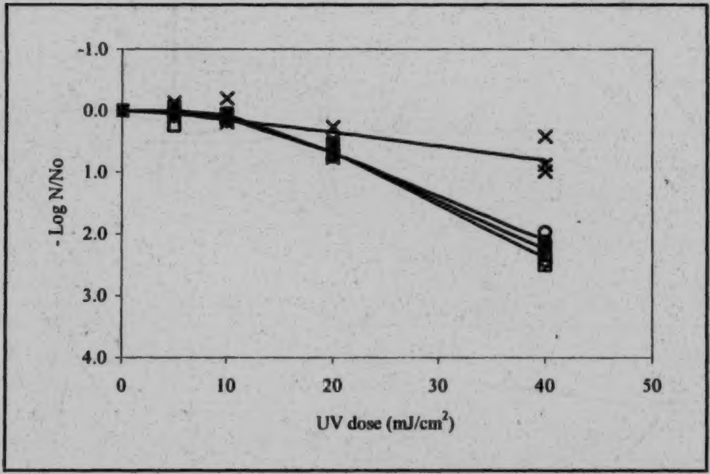

Figure 4-14 Comparison between UV inactivation of spores present in filtered water that was homogenized before (◇) and after (×) UV exposure, spores added directly to filtered water (○) and spores in phosphate buffered DI water (□).

From Figure 4-14, it can be seen that there is a difference between the inactivation curves of samples that were homogenized after UV exposure compared to the inactivation curves of samples that were homogenized before UV exposure. In contrast, there was a good agreement between the inactivation of spores added to filtered water, the inactivation of spores added to particle-free phosphate buffered DI water, and the inactivation of spores present in filtered water that was homogenized before UV exposure. An ANOVA test indicated that there was a statistical difference (*p*-value = 1.3×10^{-9}, n = 48). Scheffe's LSD test indicated that, at the 95% confidence level and at 40 mJ/cm^2, the only statistical differences were between mean inactivation of spores in the filtered water that was homogenized after UV exposure and the other three treatments. This result not only supports the hypothesis that adding microorganisms into filtered water containing particulate matter may not account for the effects of aggregation but is another indication that microorganisms that broke through the filter media were aggregated.

4.4 DIRECT MEASUREMENT OF AGGREGATION

In order to directly measure aggregation, filtered water was passed through a series (10, 5, 2, and 0.4 μm) of polycarbonate track etched membrane filters. This serial filtration experiment was conducted only in Phase 2. The number distributions of four replicated measurements of particle-size in the composite filtered effluent sample are shown in Figure 4-15 in accordance with Equation 4-1 (Lawler et al. 1980). Particle and microbial counts for this section are presented in Table D-5 Appendix D.

$$\frac{dN_p}{d(\log d_p)} = 2.3 d_p n_p(d_p), \qquad \text{Equation 4-1}$$

where N_p is the total number of particles, d_p is the particle diameter (μm), and $n_p(d_p)$ is the number distribution of particles (number/cm^{3}μm).

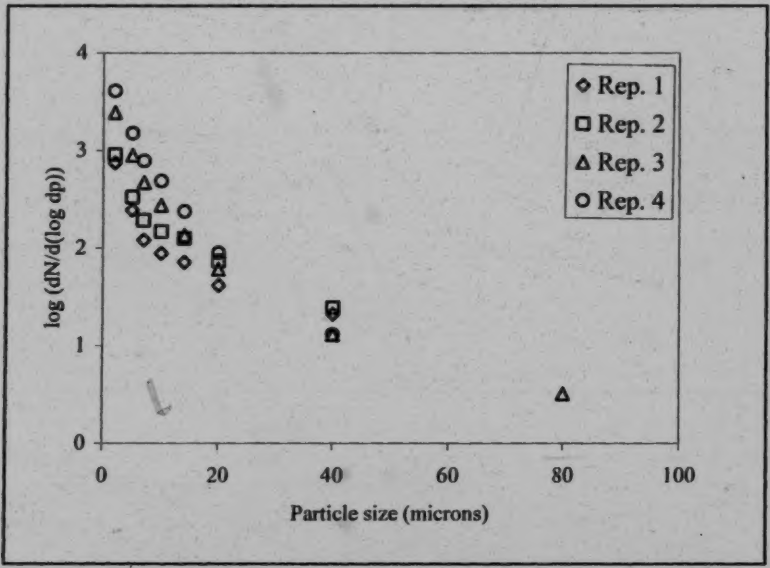

Figure 4-15 Number distribution of particles in filtered water. Four replicated measurements (Reps 1 to 4) of the particles in the filtered water were done using the Hiac-Royco Particle Counting System.

As can be seen from Figure 4-15, filtered water that broke through the filter media had particles that were up to 20 μm in size even though a few particles up to 80 μm size range were counted. Particles large enough to engulf or shield spores from UV radiation, therefore, were detected in the filtered water. Table 4-6 shows the effect of sample homogenization on the determination of the spore concentration in the filter effluent. The concentration of spores captured on each filter was then represented as a fraction of the total spore concentration as enumerated in the homogenized filter effluent (i.e. 1.82 x 10^4 CFU/mL). This concentration was then used to compute the percentage of spores retained on the different cut-off sizes. The results of this computation, presented in Table 4-6, provide a direct measure of the level of spore aggregation in the filtered effluent. For example, on average, 1.3% of the spores present in the filtered water were captured on the 10 μm filter. Because of this finding, it was hypothesized that spores may be associated with particles that were greater than 10 μm in size. Similarly, 1.8% of spores in filtered water were associated with particles in the 5 to 10 μm size range and 4.7% of spores were associated with particles in the 2 to 5 μm size range. The total of the average result associated with each particle-size range was only 24%, which is much less than the theoretical value of 100%.

Table 4-6 Results of *B. subtilis* spore enumeration from particulate material captured on PCTE filters after serial filtration of the filter effluent. (Note: all samples were homogenized prior to enumeration)

Filter size (µm)	Size fraction (µm)	Replicate	Total spore population retained on filter[a] (%)	Total spore population retained on filter[b] (%)
10	≥ 10	1	0.80	
		2	0.84	
		3	2.22	
		Average[c]	1.3 ± 0.9	5.4 ± 3.9
5	≥ 5 ≤ 10	1	1.49	
		2	2.48	
		3	1.03	
		4	2.00	
		Average[c]	1.8 ± 0.6	7.3 ± 2.6
2	≥ 2 ≤ 5	1	2.64	
		2	5.16	
		3	6.18	
		Average[c]	4.7 ± 0.5	19.4 ± 8.8
0.4	≥ 0.4 ≤ 2	1	18.9	
		2	13.9	
		3	16.2	
		Average[c]	16.3 ± 2.9	68.0 ± 12

[a] relative to the total number of spores enumerated in homogenized samples of the filter effluent

[b] relative to the total number spores captured on the all filters

[c] arithmetic mean ± 2 × std. error of three CFU determinations

The relatively low overall recovery on the PCTE filters may have been due to the incomplete removal of the particles from the filter surface or the presence of filter pore openings larger than the rated nominal pore size. The incomplete removal of spores might have resulted because spores were either stuck in the filter pores or remained on the surface after washing even though the membrane filter was soaked in phosphate buffered DI water for up to 30 min before washing commenced. Microscopic examination of the PCTE filters revealed the presence of openings considerably larger than the nominal filter pore size due to imperfections in the track-etching process (Section 4.5.1). More examples of pores larger than the rated nominal pore sizes are presented in Section 4.5 and Appendix E. Because the spores used in this study were approximately 1.4 to 1.7 µm in length, it was expected that the individual spores that passed through 10, 5, and 2 µm would be caught on the 0.4 µm membrane filter. In Figure 4-16, for example, four pores were merged into one opening of approximately 1.6 µm on a 0.4 µm filter. Therefore, spores may have been lost through such openings. This explanation is supported by the presence of particles that were greater than 2 µm in the filtrate of the 0.4 µm membrane filter. Hence, particles that were greater than the nominal size of the membrane filter passed through the filters. In Tables D-1 to D-4 and Figures D-1 to D-4 in Appendix D, particles counts and number distribution of particles that passed through each filter size are provided, respectively.

When the results of Table 4-6 were normalized against the total number of spores recovered on all filters, the percentage of spores in the sample that were aggregated with particles greater than 10 µm in size was 5.4% and the percentage that were associated with particles that were greater than 5 µm but less that 10 µm was 7.3%. However, the recovery of spores from the membrane filter may be unequal; therefore, the normalized percentages may not be an accurate representation of the actual percentage of spores retained on each filter. Therefore, the actual percentage of the total spore population on each filter was taken to be a better representation of the aggregation in filtered water. Nevertheless, the PCTE filters provided evidence of aggregation and particle-association.

4.5 MICROSCOPIC EXAMINATION OF AGGREGATION

SEM was used to examine the surface of the polycarbonate track etched membrane filters used in the serial filtration of the filtered water. The objectives of the SEM analysis were to examine the effect of homogenization of *B. subtilis* spore suspension, to determine if a cake had developed, and to determine the characteristics of aggregated spores. The washing and dehydration steps used in the preparation of samples prior to gold sputtering may have created experimental artifacts. Because the membrane filters were fixed with 2% glutaraldehyde immediately after serial filtration of the filtered water, artifacts resulting from washing and dehydration were expected to be minimal (personal communication with Jack Scott, Advanced Microscopy Unit, Dept. of Biological Science, University of Alberta).

4.5.1 Effects of Homogenization

In Section 4.1.5, it was determined that the there was a statistical difference between enumerated spore concentrations in homogenized and non-homogenized samples. A scanning electron microscope was then used to visually examine the effect of homogenization on the spore suspension, as well as visible damage to the coat of individual spores. In Figures 4-17, it can be seen that the non-homogenized spore suspension contained some aggregated spores. Spores in the homogenized suspensions (Figure 4-18) were more dispersed than those in the non-homogenized suspensions (Figure 4-17). These SEM images (Figures 4-16 and 4-17) are consistent with the increase in the enumerated spore concentration that accompanied homogenization in the UV exposure trials (Sections 4.3.1 and 4.3.2). This finding also provides further evidence that the homogenization step broke up aggregated spores in the filtered and backwash water. Concerns about the possible effects of homogenization on individual spores were also addressed. As can be seen in Figure 4-16, there was no evidence of gross damage to the spore coat. Most spores appeared intact (Figure 4-16).

Figure 4-16 SEM micrograph showing the effect of homogenization on individual *B. subtilis* spores passed through a 0.4 μm PCTE membrane filter (Magn. = 10 000x). Circles show large openings that were observed on the filter.

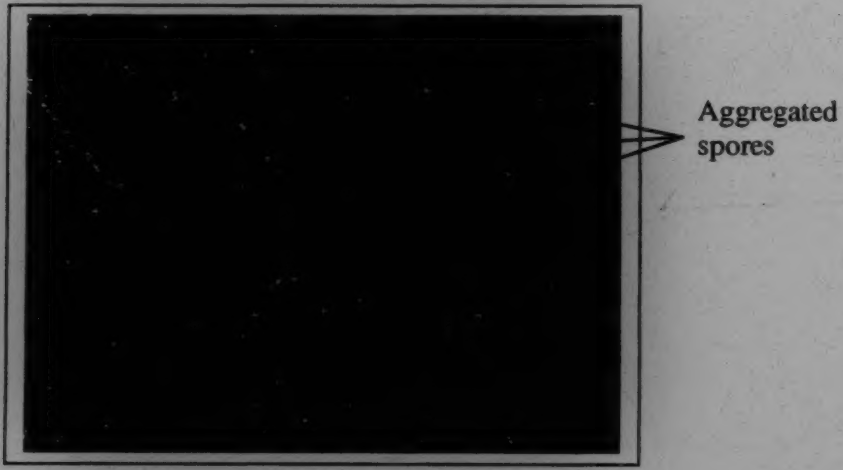

Figure 4-17 SEM micrograph showing a non-homogenized *B. subtilis* spore suspension passed through a 0.4 μm PCTE membrane filter (Magn. = 601x).

Figure 4-18 SEM micrograph showing the effect of homogenization on a *B. subtilis* spore suspension passed through a 0.4 μm PCTE membrane filter (Magn. = 601x).

4.5.2 Cake formation

A cake is a layer of solids or particles on a membrane filter. Cake formation on a PCTE membrane filter can lead to a reduction in the effective pore size. Such a reduction means that the particles and spores can be caught on filters of larger rated pore size. In this study, a scanning electron microscope was used to examine the surface of the membrane filter to find any evidence of cake formation on the filter. Figures 4-19 to 4-22 provide SEM views of typical 2 μm, 5 μm and 10 μm membrane filters after serial filtration. Little evidence was found of filter cake formation. Most of the pores appeared clear and unobstructed. This finding suggests that the effective cut-off size of the filters did not change during the serial filtration.

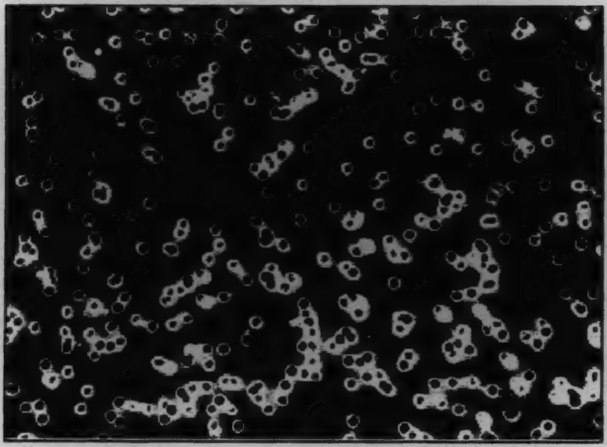

Figure 4-19 SEM micrograph showing the surface of a 2 μm PCTE membrane filter after passing filtered effluent (Magn. = 857x).

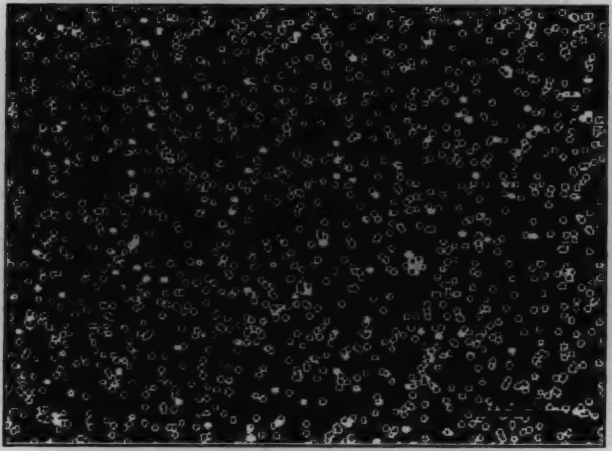

Figure 4-20 SEM micrograph showing the surface of a 5 μm PCTE membrane filter after passing filtered effluent (Magn. =156x).

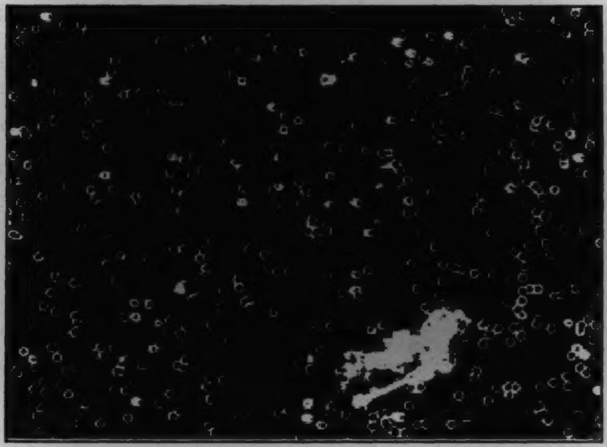

Figure 4-21 SEM micrograph showing the surface of a 5 μm PCTE membrane filter after passing filtered effluent (Magn. = 255x).

Figure 4-22 SEM micrograph showing the surface of a 10 μm PCTE membrane filter after passing filtered effluent (Magn. = 100x).

4.5.3 Characteristics of Aggregated *B. subtilis* Spores in Filtered Water

Evidence was presented (Sections 4.3, 4.4 and 4.5.1) that some *B. subtilis* spores that broke through the sand/anthracite filter were aggregated. In many previous studies, particle-association of microorganisms has been identified as the cause of the reduced effectiveness of UV inactivation (Emerick et al. 2000; Emerick et al. 1999; Jolis et al. 2001; Loge et al. 2001; Loge et al. 1996; Loge et al. 1999; Qualls et al. 1983; Qualls et al. 1985). Figures 4-23 to 4-26 reveal that spores may be attached to each other by floc rather than to particles. Figures 4-23 and 4-24 show a number of spores attached to each other whereas Figures 4-25 and 4-26 show floc of sufficient size to encapsulate and shield spores from UV radiation. However, these SEM images do not provide any evidence that these large floc particles contained spores. It was estimated from the SEM images that aggregated spores were 7 to 10 μm in size while the large floc particle was 50 to 80 μm in size.

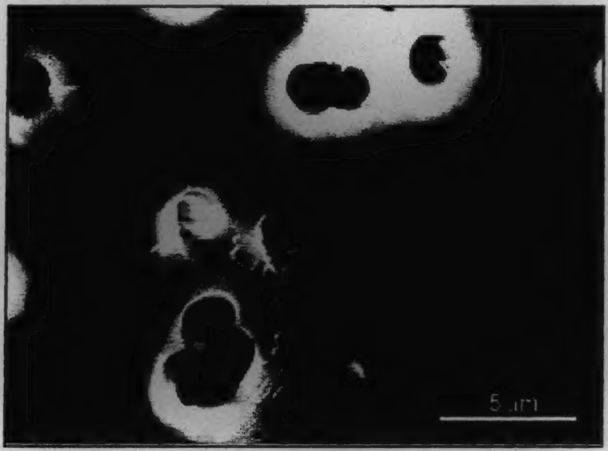

Figure 4-23 SEM micrograph showing aggregated spores detected on a 2 μm PCTE membrane filter (Magn. = 5000x).

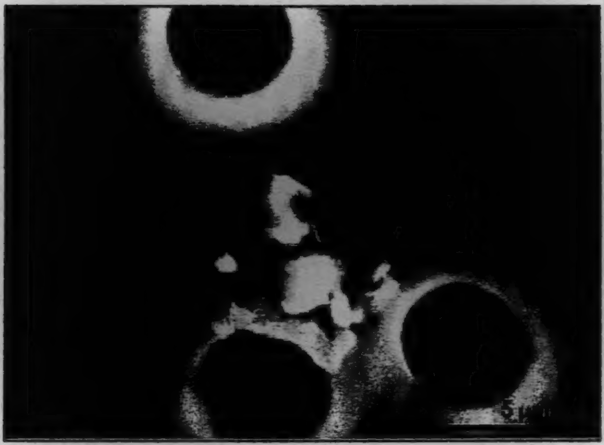

Figure 4-24 SEM micrograph showing a spore-floc aggregate detected on a 5 μm PCTE membrane filter (Magn. = 5000x).

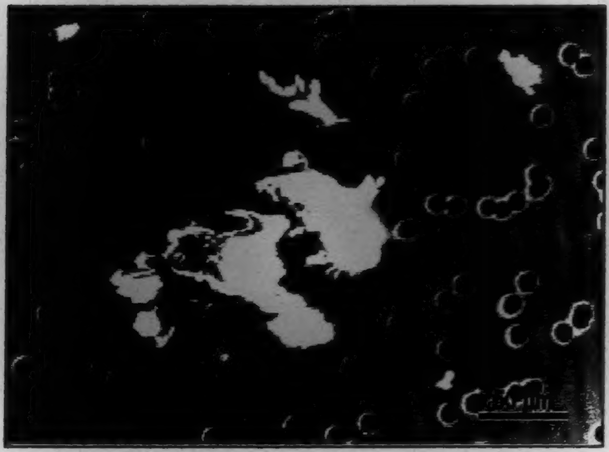

Figure 4-25 SEM micrograph showing a large particle detected on a 10 μm PCTE membrane filter (Mang. = 314x).

Figure 4-26 SEM micrograph showing a large particle detected on a 10 µm PCTE membrane filter (Magn. = 200x).

5 DISCUSSION OF RESULTS

The effect of particles on the UV inactivation of microorganisms was discussed in Section 2.8. Batch et al. (2004) and Passantino et al. (2004) concluded that the particles added directly to filtered and unfiltered water had no effect on the UV inactivation of microorganisms as long as the effect of reduced transmittance was factored into the dose calculation. Harrington et al. (2003) indicated that the state of aggregation of microorganisms that broke through a filtration media was unknown. As well, many waterborne outbreaks are known to have occurred during periods of sub-optimal operations of the filtration process (Hrudey and Hrudey 2004). In some cases, the failure of treatment systems to act as a barrier resulted in the outbreak to pathogenic microorganisms. Hence, the objectives of this study were: to determine the extent of microorganism aggregation in filtered and backwash water, to determine the effect of aggregation on the UV inactivation of surrogate microorganisms (*B. subtilis*) in filtered water, to directly measure aggregation through isolation using a series of filters, and to visually examine the aggregation using an electron microscope. The objectives of this study tested the hypothesis that microorganisms that broke through a filtration media under sub-optimal treatment conditions would be aggregated, and that the UV inactivation of these aggregated microorganisms would be reduced.

Earlier studies on UV disinfection from which the *I*t* table in the UVDGM was formulated did not account for the effects of aggregation generated upstream of the UV reactor. The *I*t* table, therefore, assumes that UV disinfection is independent of upstream treatment processes. In this study, when a laboratory-scale media filter was operated sub-optimally, a fraction of the spores that broke through the filter were found to be aggregated. A homogenization step broke up aggregated spores in filtered and backwash water to create the several treatment conditions. It should be noted, however, that homogenization was purely an experimental technique used to observe the effects of aggregation. In water treatment systems, feed water to the UV reactors is not homogenized. Little vigorous mixing of the water occurs downstream of the filters, and microbe-particle aggregates are unlikely to disintegrate prior to UV reactors. The homogenization step used in this study would be difficult to implement in water treatment plants because of high flow rates, large volume, and high energy required to achieve the same extent of mixing. Nonetheless, aggregated spores (or pathogens) would behave like dispersed spores or pathogens in terms of disease-causing potential in an exposed host.

Different disinfection performance scenarios can be related to the characteristics of the experimental treatment conditions used in this study. Because homogenization broke apart aggregates, the spores in backwash and filtered water samples homogenized before UV exposure were more likely to be mono-dispersed. Thus, the spores in this experimental condition will be more susceptible to UV inactivation. The inactivation of spores resulting from such treatment condition depicts the "best case" performance of UV disinfection systems. The inactivation of spores in particle-free phosphate buffered DI water may represent an "ideal" UV disinfection performance. However, the inactivation results obtained from samples homogenized after UV exposure is likely to be the best predictor of the true "in-plant" UV disinfection performance. Non-homogenized samples may have similar UV inactivation characteristics to those of samples homogenized after UV exposure. This experimental treatment condition, which represents a case where homogenization is not implemented (or microorganisms are not mono-dispersed), as is usually the case in many high flow UV reactors, can be described as the "measurable" UV disinfection performance.

The interpretation of the results of this study in terms of UV disinfection performance will provide useful information on the effects of aggregation on UV disinfection of microorganisms. In backwash water, a significant difference was found between the inactivation of spores in phosphate buffered DI water and the inactivation of spores contained in samples that were homogenized before UV exposure. The presence of floc material in backwash water may have shielded spores from UV radiation, or the dispersion of spores in backwash water may not have been 100% efficient. In Phase 1, the inactivation rate constant, k_m, was observed to have been underestimated by a factor of 3.6 and 1.3 for the backwash and filtered water, respectively, when the true in-plant performance was compared to the best case performance. Phase 1 results were similar to those obtained in Phase 2. For Phase 2 backwash water samples, the inactivation rate constant, k_m, was underestimated by a factor of 2.7, 3.6 and 1.0 when that true in-plant performance was compared to the best case, ideal and measurable performance, respectively. Like with backwash water, the inactivation rate constant, k_m of spores in filtered water was underestimated by a factor of 3.5, 3.6 and 3.3 when the true in-plant performance was compared to the best case, ideal, and measurable performance, respectively. In addition, no significant difference was found between the inactivation rate constants of the true in-plant and the measurable performance for the Phase 2 experiments. At low doses, up to 10 mJ/cm^2, there was no significant difference in the inactivation of spores among the measurable, best case and true in-plant disinfection performance. One possible explanation is that the presence of mono-dispersed spores that may have been inactivated at low UV doses. At higher doses, complete enmeshment may have been responsible for the limited inactivation of spores. The underestimated UV inactivation rate constants suggest the need to take the aggregation into account during the design of UV disinfection systems. This finding also suggests the dependence of disinfection systems on the performance of upstream treatment processes.

The serial filtration was able to assess the extent of aggregation that resulted in the limited disinfection performance. About 1.3% and 1.8% of spores in the filtered water were in aggregates of greater or equal to 10 µm and 5 to 10 µm size range, respectively. Given that the spores used in this study were rods in the 1.4 to 1.8 µm in size and the particle-size distribution of the filtered water, the potential existed for shielding and enmeshment of spores into microbe-microbe and microbe-particle aggregates. The presence of these aggregates may have been responsible for the limited disinfection performance measured. The presence of aggregates that could shield UV radiation implies that there is a possibility that sub-optimally operated treatment systems could reduce the effectiveness of UV reactors. These results are consistent with the hypothesis that the presence of aggregates would affect the UV inactivation of microorganisms.

At this point, aggregation, enmeshment and particle-association must be distinguished from each other according to the results of this study. "Aggregates" can be described as individual microorganisms massed into a dense cluster. "Enmeshment," on the other hand, is the complete covering or coating of microorganisms by a particulate material. "Particle-association" can be described as the attachment of particles and microorganisms to each other, including surface attachment and aggregation. The SEM images revealed that "aggregation" may be a better term to describe the relationship between spores because some evidence of floc holding spores in clusters was found on some SEM images (Figures 4-23 and 4-24). In other cases, floc particles that were large enough to enmesh two or more spores were identified (Figures 4-25 and 4-26) even though it was not quantitatively determined whether spores were enmeshed in these large particles. Hence, "aggregation" and "enmeshment" are better terms than "particle-association" for describing the aggregating effects of upstream treatment processes on microorganisms. The SEM results supported the hypothesis that a fraction of the spores that broke through a sub-optimally operated media filter were aggregated; and that aggregation had an effect on the UV inactivation of spores in filtered water. The findings from the UV inactivation of spores in filtered and backwash water, serial filtration and electron microscopy supported each other and were consistent with the hypothesis of this study.

However, this result is in contrast to the results of earlier studies on the effects of particle-association on the UV inactivation of microorganisms in filtered and unfiltered water (Batch et al. 2004; Passantino et al. 2004). One possible explanation for the insignificant effects in those studies is that the particles and microorganisms used in these studies were added directly to filtered water. Hence, the microorganisms had little opportunity to be aggregated. No significant difference was observed between the inactivation of spores dispersed in DI water and the inactivation of spores added to filtered water. This finding supports the earlier results obtained by Batch et al. (2004) and Passantino et al. (2004) because light scattering was taken into account during dose determination in those studies as well as in this present study. Amoah et al. (2005) found a modest reduction in the inactivation of seeded *C. parvum* oocysts and *G. muris* cysts in lake sediments suspensions when the turbidity was increased from 0.3 to 20 NTU and 7.5 to 20 NTU, respectively. This modest difference may have resulted from the minimal aggregation or surface attachment of *C. parvum* oocysts and *G. muris* cysts. Coagulated humic and activated sludge particles added to a synthetic water matrix had a protective effect on viral surrogates (Templeton et al. 2005). Synthetic water may not represent natural raw water, nor does it represent secondary effluent, but useful insights into the size of aggregated viral particles and the possible protective effect of humic and activated sludge particles were highlighted.

Despite variations in the river water and settled water quality used in two phases of this study, the results obtained were reproducible. The two filtration runs done in this study had similar loading to those obtainable in full scale water treatment systems. Therefore, aggregates from the filter column may be similar to the aggregates from a filter bed if operated at sub-optimal conditions. Because the inactivation of spores in Phase 1 was consistent with the inactivation of spores Phase 2, the characteristics of the water matrix may be similar in both phases. It was also observed that the inactivation of spores in non-homogenized samples was consistently greater than the inactivation of spores in samples homogenized after UV but consistently less than the inactivation of spores in samples homogenized before UV exposure. In Phase 1 and Phase 2 filtered water experiments, for example, the inactivation rate constant was underestimated by factors of 3.6 and 3.5, respectively, when the true in-plant performance was compared to the best case performance. These findings demonstrate that, for comparable experimental treatment conditions, the inactivation of spores were consistent.

Wall effects, floc characteristics and sampling are some possible limitations that may affect the results obtained in this study. A 2.5 cm column will definitely have a larger surface area to volume ratio than those of a filter bed. Thus, the wall effects may be different in these two circumstances as a result of the scale down. Therefore, the results of this study may have been influenced by some unknown factors as a result of the wall effects. In order to account for this effect, there might be need to further investigate the result of this study with larger size filter columns. Alum floc may have different characteristics than iron floc. The differences that may result from using a different coagulant or polymer were not investigated in this study. It may be worthwhile to know whether the type of coagulant used might make any difference to the results, especially the particle size distribution. Filtered and backwash water samples were collected as a composite sample. Spores collected from different regime of the filtration run may have different characteristics. It may be that mono-dispersed spores will be dominant in the early part of the filtration run while aggregates may break through afterwards. If water samples are collected from different regimes of the filtration run, say ripening, filtration or break through, the results obtained may be different than those obtained in this study.

6 CONCLUSIONS AND RECOMMENDATIONS

Particles in water samples have been known to have an effect on the UV inactivation of microorganisms. Moreover, many waterborne outbreaks have been associated with periods of sub-optimal performance of treatment systems. A combination of these two effects may have implication for UV disinfection of drinking water especially, when UV reactors are most needed (when the treatment systems are operating under sub-optimal treatment conditions).

6.1 CONCLUSIONS

At the end of this research, the following conclusions were reached:

1. Some fraction of the microorganisms that break through operating dual media filters during conditions of sub-optimal coagulation are likely to be aggregated, either as microbe-microbe or microbe-particle aggregates. This aggregation was demonstrated by using polycarbonate track-etched filters and was confirmed by SEM. Further, this aggregation may have had a measurable and statistically significant effect on the apparent UV inactivation of microorganisms in the filtered water. The same phenomenon was observed in filter backwash water, but to a greater extent. These findings could have implications for the dose requirements of UV reactors intended for the primary disinfection of filtered drinking water.

2. The inactivation rate constant of *B. subtilis* spores in filtered and backwash water was underestimated as a result of aggregation. The results of this study suggest that the measurable performance of a UV system may underestimate the true performance when aggregated microorganisms are present in filtered drinking water. The extent of inactivation rate constant underestimation may vary with the raw water quality and the extent of aggregation.

3. Adding microorganisms directly into filtered water containing particulate matter may not account for the effects of aggregation on the UV inactivation of microorganisms in filtered water.

6.2 RECOMMENDATIONS

Some recommendations as a result of this study are

1. The findings of this study highlight the importance of maintaining properly optimized upstream processes.

2. Because the aggregation is currently not considered in the *I*t* table provided in the UV guidance manual, it is recommended that a factor of safety be applied to the rate constant used for the design of future UV reactors. Although increasing UV dose may not inactivate microorganisms that are completely engulfed but there is need to recognize that the current values in the *I*t* table need to account for aggregation. In addition, maintaining a residual disinfectant in the distribution system is still very important.

3. The microscopic examination of spores on membrane filters revealed that spores were aggregated to each other by floc materials. This finding may be a possible reason for the difference between the UV inactivation of non-homogenized samples and samples that were homogenized before and after UV exposure. Hence, further investigations into the ability of UV radiation to penetrate floc material should be considered.

4. Other types of coagulants and polymers could be studied to determine if a change in the coagulant or polymer type would have any impact on the results of this study. In addition, different microorganisms including viruses could be studied under varying turbidity conditions in a flow through system.

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APPENDIX A EXPERIMENTAL DATA AND INFORMATION FROM CONTROL EXPERIMENTS

A-1 EXPERIMENTAL DATA

Table A- 1 Counts of indigenous spores in raw water used for Phase 1 and Phase 2 experiments. Samples were pasteurized at 75°C for 15 min before incubation at 37°C for 48 h.

Phase	Samples	Dilution	Counts			Average CFU/mL
			1	2	3	
1	Sample 1	0	6	12	1	6
	Sample 2	0	9	7	8	8
2	Sample 1	0	7	9	11	9
	Sample 2	0	7	6	2	5
			Average			**7 CFU/mL**

Experimental data and information related to the effect of post UV exposure pasteurization can be found in Table B-6 and Table B-7.

Tables A-2 to A-11 were used to plot Figures 4-3 to 4-4

Table A- 2 Colony counts for the impact of homogenization on spores viability at 0 h.

Treatment	Dilution	Count			Average CFU/mL
		1	2	3	
Not Homogenized	10^7	60	73	58	64 x 10^7.
Homogenized	10^7	81	80	87	83 x 10^7

Table A- 3 Colony counts for the impact of homogenization on spore viability at 24 h. Two 10 mL sub-samples were taken from *homogenized B. subtilis* suspension and was either homogenized or not homogenized.

Treatment	Dilution	Count			Average CFU/mL
		1	2	3	
Not Homogenized	10^{-7}	43	56	61	53 x 10^{7}
	10^{-8}	11	9	12	11 x 10^{7}.
Homogenized	10^{-7}	79	74	82	78 x 10^{7}
	10^{-8}	4	4	1	30 x 10^{7}

Table A- 4 Colony counts for the impact of homogenization on spore viability at 24 h. Two 10 mL sub-samples were taken from *non-homogenized B. subtilis* suspension and was either homogenized or not homogenized.

Treatment	Dilution	Count			Average CFU/mL
		1	2	3	
Not Homogenized	10^{-7}	55	64	75	65 x 10^{7}
	10^{-8}	8	7	7	73 x 10^{7}
Homogenized	10^{-7}	64	62	60	62 x 10^{7}
	10^{-8}	9	10	12	10 x 10^{8}

Table A- 5 Colony counts for the impact of homogenization on spore viability at 24 h. Two 10 mL sub-samples were taken from homogenized *B. subtilis* suspension and was homogenized every 24 h.

Treatment	Dilution	Count			Average CFU/mL
		1	2	3	
Homogenized	10^{-7}	74	59	77	70 x 10^{7}
	10^{-8}	10	9	14	11 x 10^{8}

Table A- 6 Colony counts for the impact of homogenization on spore viability at 48 h. Two 10 mL sub-samples were taken from *homogenized B. subtilis* suspension and was either homogenized or not homogenized.

Treatment	Dilution	Count			Average
		1	2	3	CFU/mL
Not Homogenized	10^{-7}	74	82	65	74×10^{7}
	10^{-8}	6	8	7	70×10^{7}
Homogenized	10^{-7}	84	81	85	83×10^{7}
	10^{-8}	8	14	9	10×10^{8}

Table A- 7 Colony counts for the impact of homogenization on spore viability at 48 h. Two 10 mL sub-samples were taken from *non-homogenized B. subtilis* suspension and was either homogenized or not homogenized.

Treatment	Dilution	Count			Average
		1	2	3	CFU/mL
Not Homogenized	10^{-7}	85	86	77	83×10^{7}
	10^{-8}	12	6	10	93×10^{7}
Homogenized	10^{-7}	76	58	66	67×10^{7}
	10^{-8}	7	8	7	73×10^{7}

Table A- 8 Colony counts for the impact of homogenization on spore viability at 48 h. Two 10 mL sub-samples were taken from homogenized *B. subtilis* suspension and was homogenized every 24 h.

Treatment	Dilution	Count			Average
		1	2	3	CFU/mL
Homogenized	10^{-7}	78	63	64	68×10^{7}
	10^{-8}	7	12	8	9×10^{7}

Table A- 9 Colony counts for the impact of homogenization on spore viability at 72 h. Two 10 mL sub-samples were taken from *homogenized B. subtilis* suspension and were either homogenized or not homogenized..

Treatment	Dilution	Count			Average
		1	2	3	CFU/mL
Not Homogenized	10^{-7}	66	83	75	75 x 10^7
	10^{-8}	10	6	11	90 x 10^7.
Homogenized	10^{-7}	62	56	51	56 x 10^7
	10^{-8}	10	7	12	97 x 10^7

Table A- 10 Colony counts for the impact of homogenization on spore viability at 72 h. Two 10 mL sub-samples were taken from *non-homogenized B. subtilis* suspension and were either homogenized or not homogenized.

Treatment	Dilution	Count			Average
		1	2	3	CFU/mL
Not Homogenized	10^{-7}	81	83	69	78 x 10^7
	10^{-8}	3	10	8	70 x 10^7
Homogenized	10^{-7}	78	78	89	82 x 10^7
	10^{-8}	7	9	12	93 x 10^7

Table A- 11 Colony counts for the impact of homogenization on spores viability at 72 h. Two 10 mL sub-samples were taken from homogenized *B. subtilis* suspension and was homogenized every 24 h.

Treatment	Dilution	Count			Average
		1	2	3	CFU/mL
Homogenized	10^{-7}	85	77	75	79 x 10^7
	10^{-8}	9	4	6	63 x 10^8

A-2 SCHEFFE'S LEAST SIGNIFICANT DIFFERENCE TEST CALCULATIONS

From a two-factor ANOVA with replication conducted on one preliminary and two final experimental data, it was found that

$MS_E = 0.01129$

$n_i = n_j = 3$

$n = 36$

$t_s = 4$

$k_s = \frac{t_s(t_s - 1)}{2} = 6$

Applying Scheffe's LSD test,

$$LSD_{ij}(S) = \sqrt{(t_s - 1)F_{(\alpha, t_s - 1, n - t_s)}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)MS_E} \qquad \text{Equation C-1}$$

$$LSD_{ij}(S) = \sqrt{3 * 2.904 * \tfrac{2}{3} * 0.1129} = 0.256$$

Table A- 12 Result of Scheffe's least significant difference test

pairs	treatments			
	5	10	20	40
$\bar{y}_1 - \bar{y}_2$	NS	S	S	S
$\bar{y}_1 - \bar{y}_3$	NS	S	S	S
$\bar{y}_2 - \bar{y}_3$	NS	NS	NS	S

NS – Not Significant

S - Significant

APPENDIX B EXPERIMENTAL DATA AND INFORMATION FROM PHASE 1 EXPERIMENTS

Table B-1 Variation of turbidity of filter effluent with time. These data were plotted in Figure 4-6.

Time (min)	Turbidity NTU
3	2.5
8.5	1.8
14	1
20.5	0.9
30	1.2
60	1.3
130	0.89
185	1.03
240	0.9
360	1

Table B-2 Data for UV inactivation of *B. subtilis* spores in filtered water that was homogenized *after* UV exposure. Data is plotted in Figure 4-9.

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
1	0	1.E-02	54	50	53	52.33	5.23E+03	
		1.E-03	7	4	5	5.33		No =
2		1.E-02	97	114	119	110.00	1.10E+04	8.23E+03
		1.E-03	10	6	7	7.67		
3		1.E-02	76	77	101	84.67	8.47E+03	
		1.E-03	9	9	11	9.67		
4	5	1.E-01	TNTC	TNTC	TNTC			
		1.E-02	36	34	40	36.67	3.67E+03	0.351
5		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	46	47	47	46.67	4.67E+03	0.247
6		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	44	43	49	45.33	4.53E+03	0.259
7	10	1.E+00	TNTC	TNTC	TNTC			
		1.E-01	142	123	162	142.33	1.42E+03	0.762
8		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	129	139	148	138.67	1.39E+03	0.774
9		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	150	150	154	151.33	1.51E+03	0.736
10	20	1.E+00	193	200	211	201.33	2.01E+02	1.612
		1.E-01	31	20	25	25.33		
11		1.E+00	161	191	193	181.67	1.82E+02	1.656
		1.E-01	22	27	28	25.67		
12		1.E+00	166	195	220	193.67	1.94E+02	1.629
		1.E-01	20	21	22	21.00		
13	40	1.E+00	26	27	36	29.67	2.97E+01	2.443
		1.E-01	2	2	1	1.67		
14		1.E+00	23	8	19	16.67	1.67E+01	2.694
		1.E-01	0	0	0	0.00		
15		1.E+00	9	6	5	6.67	6.67E+00	3.092
		1.E-01	0	0	0	0.00		

Table B- 3 Data for UV inactivation of *B. subtilis* spores in filtered water that was homogenized *before* UV exposure. Data is plotted in Figure 4-9.

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
1	0	1.E-02	TNTC	TNTC	TNTC			
		1.E-03	25	18	17	20.00	2.00E+04	
2		1.E-02						2.21E+04
		1.E-03	23	15	17	18.33	1.83E+04	
3		1.E-02						
		1.E-03	24	27	33	28.00	2.80E+04	
4	5	1.E-02	75	76	115	88.67	8.87E+03	0.397
		1.E-03	8	10	10	9.33		
5		1.E-02	81	81	89	83.67	8.37E+03	0.422
		1.E-03	9	12	15	12.00		
6		1.E-02	158	82	76	105.33	1.05E+04	0.322
		1.E-03	13	22	15	16.67		
7	10	1.E-01	158	170	145	157.67	1.58E+03	1.147
		1.E-02	21	47	23	30.33		
8		1.E-01	149	140	144	144.33	1.44E+03	1.185
		1.E-02	14	18	20	17.33		
9		1.E-01	227	179	169	191.67	1.92E+03	1.062
		1.E-02	29	37	31	32.33		
10	20	1.E+00	214	175	210	199.67	2.00E+02	2.044
		1.E-01	25	34	24	27.67		
11		1.E+00	170	132	134	145.33	1.45E+02	2.182
		1.E-01	16	15	17	16.00		
12		1.E+00	214	207	209	210.00	2.10E+02	2.022
		1.E-01	18	27	24	23.00		
13	40	1.E+00	5	4	5	4.67	4.67E+00	3.676
		1.E-01						
14		1.E+00	3	6	9	6.00	6.00E+00	3.566
		1.E-01						
15		1.E+00	3	3	3	3.00	3.00E+00	3.867
		1.E-01						

Table B-4 Data for UV inactivation of *B. subtilis* spores in backwash water that was homogenized *before* UV exposure. Data is plotted in Figure 4-8.

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
1	0	1.E-05	13	14	9	12.00	1.20E+06	
		1.E-06	2	2	0	1.33		
2		1.E-05	9	15	7	10.33	1.03E+06	1.14E+06
		1.E-06	2	1	0	1.00		
3		1.E-05	11	16	9	12.00	1.20E+06	
		1.E-06	2	1	0	1.00		
4	5	1.E-04	164	123	105	130.67	1.31E+06	-0.058
		1.E-05	14	9	13	12.00		
5		1.E-04	106	125	113	114.67	1.15E+06	-0.001
		1.E-05	6	15	13	11.33		
6		1.E-04	85	88	121	98.00	9.80E+05	0.067
		1.E-05	9	4	15	9.33		
7	10	1.E-03	TNTC	TNTC	TNTC			
		1.E-04	68	61	46	58.33	5.83E+05	0.293
8		1.E-04	63	74	78	71.67	7.17E+05	0.203
		1.E-05	8	4	3	5.00		
9		1.E-04	67	70	73	70.00	7.00E+05	0.213
		1.E-05	6	10	6	7.33		
10	20	1.E-03	143	155	140	146.00	1.46E+05	0.894
		1.E-04	13	23	16	17.33		
11		1.E-03	128	128	122	126.00	1.26E+05	0.958
		1.E-04	15	14	12	13.67		
12		1.E-03	164	158	160	160.67	1.61E+05	0.853
		1.E-04	13	14	10	12.33		
13	40	1.E-02	46	37	42	41.67	4.17E+03	2.439
		1.E-03	4	4	2	3.33		
14		1.E-02	42	53	41	45.33	4.53E+03	2.402
		1.E-03	0	5	4	3.00		
15		1.E-02	37	33	48	39.33	3.93E+03	2.464
		1.E-03	4	2	7	4.33		

Table B- 5 Data for UV inactivation of *B. subtilis* spores in backwash water that was homogenized *after* UV exposure. Data is plotted in Figure 4-8

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
1	0	1.E-05	18	20	16	18.00	1.80E+06	
		1.E-06	2	1	4	2.33		
2		1.E-04	161	161	150	157.33	1.57E+06	1.60E+06
		1.E-05	9	13	17	13.00		
3		1.E-05	15	19	9	14.33	1.43E+06	
		1.E-06	3	2	1	2.00		
4	5	1.E-04	150	162	167	159.67	1.60E+06	0.002
		1.E-05	23	17	22	20.67		
5		1.E-04	145	140	120	135.00	1.35E+06	0.074
		1.E-05	10	16	17	14.33		
6		1.E-04	151	147	159	152.33	1.52E+06	0.022
		1.E-05	18	21	13	17.33		
7	10	1.E-04	123	119	100	114.00	1.14E+06	0.148
		1.E-05	7	13	9	9.67		
8		1.E-04	71	88	85	81.33	8.13E+05	0.294
		1.E-05	1	9	5	5.00		
9		1.E-04	183	177	198	186.00	1.86E+06	-0.065
		1.E-05	15	17	26	19.33		
10	20	1.E-04	74	71	82	75.67	7.57E+05	0.326
		1.E-05	10	5	13	9.33		
11		1.E-04	97	100	118	105.00	1.05E+06	0.184
		1.E-05	9	9	13	10.33		
12		1.E-04	61	87	83	77.00	7.70E+05	0.318
		1.E-05	9	6	5	6.67		
13	40	1.E-02	TNTC	TNTC	TNTC			
		1.E-03	382	406	428	405.33	4.05E+05	0.597
14		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	429	390	387	402.00	4.02E+05	0.600
15		1.E-03	217	186	243	215.33	2.15E+05	0.872
		1.E-04	15	15	22	17.33		

Table B- 6 Data for UV inactivation of *B. subtilis* spores that was seeded into pH 7 phosphate buffered DI water. Data is plotted in Figures 4-2, 4-8, and 4-9

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
1	0	1.E-05	27	32	21	26.67	2.67E+06	
		1.E-06	2	2	2	2.00		
2		1.E-05	40	30	30	33.33	3.33E+06	3.00E+06
		1.E-06	7	1	4	4.00		
3		1.E-05	31	28	31	30.00	3.00E+06	
		1.E-06	3	2	9	4.67		
4	5	1.E-04	241	248	258	249.00	2.49E+06	0.081
		1.E-05	25	18	23	22.00		
5		1.E-04	270	246	242	252.67	2.53E+06	0.075
		1.E-05	19	23	25	22.33		
6		1.E-04	215	201	216	210.67	2.11E+06	0.154
		1.E-05	25	35	20	26.67		
7	10	1.E-04	121	129	140	130.00	1.30E+06	0.363
		1.E-05	16	10	8	11.33		
8		1.E-04	143	139	135	139.00	1.39E+06	0.334
		1.E-05	6	15	14	11.67		
9		1.E-04	115	125	99	113.00	1.13E+06	0.424
		1.E-05	10	13	9	10.67		
10	20	1.E-02	TNTC	TNTC	TNTC			
		1.E-03	150	151	133	144.67	1.45E+05	1.317
11		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	115	140	109	121.33	1.21E+05	1.393
12		1.E-03	224	221	230	225.00	2.25E+05	1.125
		1.E-04	23	19	24	22.00		
13	40	1.E-01	212	246	210	222.67	2.23E+03	3.129
		1.E-02	18	24	23	21.67		
14		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	45	37	34	38.67	3.87E+03	2.890
15		1.E-01	131	141	117	129.67	1.30E+03	3.364
		1.E-02	9	7	12	9.33		

Table B- 7 Data for UV inactivation of *B. subtilis* spores that was seeded into pH 7 phosphate buffered DI water. Exposed samples were pasteurized at 80 °C for 15 min. Data is plotted in Figure 4-2.

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
1	0	1.E-05	27	32	21	26.67	2.67E+06	
		1.E-06	2	2	2	2.00		
2		1.E-05	40	30	30	33.33	3.33E+06	3.00E+06
		1.E-06	7	1	4	4.00		
3		1.E-05	31	28	31	30.00	3.00E+06	
		1.E-06	3	2	9	4.67		
4	5	1.E-04	241	248	258	249.00	2.49E+06	0.081
		1.E-05	25	18	23	22.00		
5		1.E-04	270	246	242	252.67	2.53E+06	0.075
		1.E-05	19	23	25	22.33		
6		1.E-04	215	201	216	210.67	2.11E+06	0.154
		1.E-05	25	35	20	26.67		
7	10	1.E-04	121	129	140	130.00	1.30E+06	0.363
		1.E-05	16	10	8	11.33		
8		1.E-04	143	139	135	139.00	1.39E+06	0.334
		1.E-05	6	15	14	11.67		
9		1.E-04	115	125	99	113.00	1.13E+06	0.424
		1.E-05	10	13	9	10.67		
10	20	1.E-02	TNTC	TNTC	TNTC			
		1.E-03	150	151	133	144.67	1.45E+05	1.317
11		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	115	140	109	121.33	1.21E+05	1.393
12		1.E-03	224	221	230	225.00	2.25E+05	1.125
		1.E-04	23	19	24	22.00		
13	40	1.E-01	212	246	210	222.67	2.23E+03	3.129
		1.E-02	18	24	23	21.67		
14		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	45	37	34	38.67	3.87E+03	2.890
15		1.E-01	131	141	117	129.67	1.30E+03	3.364
		1.E-02	9	7	12	9.33		

APPENDIX C EXPERIMENTAL DATA AND INFORMATION FROM PHASE 2 UV INACTIVATION EXPERIMENTS.

Table C- 1 Data for UV inactivation of *B. subtilis* spores in backwash water that was *not homogenized.* These data were plotted in Figure 4-11.

Run No.	Dose	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
1	0	1.E-03	217	217	212	215.33	2.15E+05	
		1.E-04	24	23	30	25.67	2.57E+05	
		1.E-05	1	2	0	1.00	1.00E+05	
2		1.E-04	28	24	26	26.00	2.60E+05	3.72E+05
		1.E-05	6	12	5	7.67	7.67E+05	
		1.E-06	0	0	0	0.00	0.00E+00	
3		1.E-04	63	66	63	64.00	6.40E+05	
		1.E-05	9	5	11	8.33	8.33E+05	
		1.E-06	2	0	1	1.00	1.00E+06	
4	5	1.E-03	TNTC	TNTC	TNTC			
		1.E-04	57	64	57	59.33	5.93E+05	-0.203
		1.E-05	2	7	6	5.00	5.00E+05	
5		1.E-04	27	26	26	26.33	2.63E+05	0.150
		1.E-05	0	4	2	2.00	2.00E+05	
		1.E-06	0	0	0	0.00	0.00E+00	
6		1.E-04	25	17	23	21.67	2.17E+05	0.234
		1.E-05	3	2	1	2.00	2.00E+05	
		1.E-06	0	0	0	0.00	0.00E+00	
7	10	1.E-03	181	168	180	176.33	1.76E+05	0.324
		1.E-04	14	16	12	14.00	1.40E+05	
		1.E-05	2	1	3	2.00	2.00E+05	
8		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	97	97	106	100.00	1.00E+05	0.570
		1.E-04	6	17	12	11.67	1.17E+05	
9		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	40	39	35	38.00	3.80E+05	-0.010
10	20	1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	30	30	23	27.67	2.77E+05	0.128
11		1.E-03	192	228	202	207.33	2.07E+05	0.254
		1.E-04	19	21	30	23.33	2.33E+05	
		1.E-05	4	3	0	2.33	2.33E+05	
12		1.E-03	208	235	208	217.00	2.17E+05	0.234
		1.E-04	25	29	27	27.00	2.70E+05	
		1.E-05	3	3	0	2.00	2.00E+05	
13	40	1.E-02	TNTC	TNTC	TNTC			
		1.E-03	157	172	184	171.00	1.71E+05	0.337
		1.E-04	24	12	16	17.33	1.73E+05	
14		1.E-02	166	163	198	175.67	1.76E+04	1.326
		1.E-03	15	18	14	15.67	1.57E+04	
		1.E-04	4	4	2	3.33	3.33E+04	
15		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	49	50	41	46.67	4.67E+04	0.901
		1.E-04	6	7	7	6.67	6.67E+04	

Table C- 2 Data for UV inactivation of *B. subtilis* spores seeded into pH 8 phosphate buffered DI water. These data were plotted in Figure 4-1.

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
16	0	1.E-03	TNTC	TNTC	TNTC			
		1.E-04	129	129	122	126.67	1.27E+06	
		1.E-05	12	19	20	17.00	1.70E+06	
17		1.E-02	TNTC	TNTC	TNTC			1.41E+06
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	146	121	143	136.67	1.37E+06	
18		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	162	162	157	160.33	1.60E+06	
19	5	1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	118	124	128	123.33	1.23E+06	0.059
20		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	128	143	149	140.00	1.40E+06	0.004
		1.E-05	16	14	11	13.67	1.37E+06	
21		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	131	133	149	137.67	1.38E+06	0.011
22	10	1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	126	99	127	117.33	1.17E+06	0.080
23		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	112	110	108	110.00	1.10E+06	0.109
		1.E-05	18	15	16	16.33	1.63E+06	
24		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	106	129	116	117.00	1.17E+06	0.082
25	20	1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	35	33	30	32.67	3.27E+05	0.636
26		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	22	27	28	25.67	2.57E+05	0.741
27		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	318	289	278	295.00	2.95E+05	0.680
28	40	1.E-01	TNTC	TNTC	TNTC			
		1.E-02	108	123	102	111.00	1.11E+04	2.105
		1.E-03	16	19	16	17.00	1.70E+04	
29		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	130	130	129	129.67	1.30E+04	2.037
		1.E-03	10	13	17	13.33	1.33E+04	
30		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	115	138	147	133.33	1.33E+04	2.025

Table C- 3 Data for UV inactivation of *B. subtilis* spores in filtered water that was *homogenized before* UV exposure. These data were plotted in Figures 4-12, 4-13, and 4-14.

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
31	0	1.E-01	TNTC	TNTC	TNTC			
		1.E-02	120	114	103	112.33	1.12E+04	
		1.E-03	10	16	9	11.67	1.17E+04	
32		1.E-02	157	130	135	140.67	1.41E+04	1.43E+04
		1.E-03	19	12	14	15.00	1.50E+04	
		1.E-04	0	3	0	1.00	1.00E+04	
33		1.E-02	175	172	179	175.33	1.75E+04	
		1.E-03	21	12	9	14.00	1.40E+04	
		1.E-04	0	2	3	1.67	1.67E+04	
34	5	1.E-01	TNTC	TNTC	TNTC			
		1.E-02	121	109	113	114.33	1.14E+04	0.096
		1.E-03	17	8	9	11.33	1.13E+04	
35		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	124	138	124	128.67	1.29E+04	0.045
		1.E-03	14	15	13	14.00	1.40E+04	
36		1.E-02	169	147	138	151.33	1.51E+04	-0.025
		1.E-03	10	20	21	17.00	1.70E+04	
		1.E-04	5	1	1	2.33	2.33E+04	
37	10	1.E-01	TNTC	TNTC	TNTC			
		1.E-02	99	121	94	104.67	1.05E+04	0.135
		1.E-03	7	10	9	8.67	8.67E+03	
38		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	100	102	103	101.67	1.02E+04	0.147
39		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	96	92	99	95.67	9.57E+03	0.174
		1.E-03	5	14	6	8.33	8.33E+03	
40	20	1.E+00	TNTC	TNTC	TNTC			
		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	30	30	36	32.00	3.20E+03	0.650
41		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	31	34	26	30.33	3.03E+03	0.673
42		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	29	39	34	34.00	3.40E+03	0.623
43	40	1.E+00	103	102	103	102.67	1.03E+02	2.143
		1.E-01	11	15	9	11.67	1.17E+02	
		1.E-02	1	1	2	1.33	1.33E+02	
44		1.E+00		97	86	91.50	9.15E+01	2.193
		1.E-01	13	9	7	9.67	9.67E+01	
		1.E-02	1	0	0	0.33	3.33E+01	
45		1.E+00	51	52	48	50.33	5.03E+01	2.453
		1.E-01	6	6	6	6.00		
		1.E-02						

Table C- 4 Data for UV inactivation of *B. subtilis* spores in backwash water that was *homogenized before* UV exposure. These data were plotted in Figure 4-10 and 4-11.

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
46	0	1.E-04	49	51	46	48.67	4.87E+05	
		1.E-05	6	5	2	4.33	4.33E+05	
		1.E-06	1	0	1	0.67	6.67E+05	
47		1.E-04	116	113	125	118.00	1.18E+06	7.77E+05
		1.E-05	8	9	17	11.33	1.13E+06	
		1.E-06	1	0	3	1.33	1.33E+06	
48		1.E-04	78	58	63	66.33	6.63E+05	
		1.E-05	5	8	7	6.67	6.67E+05	
		1.E-06	1	1	0	0.67	6.67E+05	
49	5	1.E-04	68	90	69	75.67	7.57E+05	0.011
		1.E-05	4	5	6	5.00	5.00E+05	
		1.E-06	0	5	0	1.67	1.67E+06	
50		1.E-04	86	100	100	95.33	9.53E+05	-0.089
		1.E-05	6	4	6	5.33	5.33E+05	
		1.E-06	0	1	1	0.67	6.67E+05	
51		1.E-04	94	99	93	95.33	9.53E+05	-0.089
		1.E-05	9	5	10	8.00	8.00E+05	
		1.E-06	0	1	0	0.33	3.33E+05	
52	10	1.E-03	TNTC	TNTC	TNTC			
		1.E-04	53	53	60	55.33	5.53E+05	0.147
		1.E-05	4	6	3	4.33	4.33E+05	
53		1.E-03	208	236	211	218.33	2.18E+05	0.551
		1.E-04	24	21	26	23.67	2.37E+05	
		1.E-05	3	3	0	2.00	2.00E+05	
54		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	52	42	33	42.33	4.23E+05	0.264
		1.E-05	8	2	3	4.33	4.33E+05	
55	20	1.E-03	TNTC	TNTC	TNTC			
		1.E-04	39	35	26	33.33	3.33E+05	0.367
		1.E-05	2	3	2	2.33	2.33E+05	
56		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	327	344	352	341.00	3.41E+05	0.357
57		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	45	42	35	40.67	4.07E+05	0.281
58	40	1.E-01	TNTC	TNTC	TNTC			
		1.E-02	83	75	86	81.33	8.13E+03	1.980
		1.E-03	7	10	7	8.00	8.00E+03	
59		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	49	28	36	37.67	3.77E+04	1.314
60		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	137	95	97	109.67	1.10E+04	1.850
		1.E-03	9	7	7	7.67	7.67E+03	

Table C- 5 Data for UV inactivation of *B. subtilis* spores in seeded filtered water. These data were plotted in Figure 4-14.

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
61	0	1.E-05	167	163	181	170.33	1.70E+07	
		1.E-06	21	27	11	19.67	1.97E+07	
		1.E-07	1	0	2	1.00	1.00E+07	
62		1.E-05	145	182	147	158.00	1.58E+07	1.54E+07
		1.E-06	12	9	16	12.33	1.23E+07	
		1.E-07	1	2	1	1.33		
63		1.E-05	136	115	146	132.33	1.32E+07	
		1.E-06	14	11	17	14.00	1.40E+07	
		1.E-07	3	2	0	1.67		
64	5	1.E-05	131	158	141	143.33	1.43E+07	0.030
		1.E-06	11	17	13	13.67	1.37E+07	
		1.E-07	1	1	2	1.33	1.33E+07	
65		1.E-05	151	131	146	142.67	1.43E+07	0.032
		1.E-06	14	15	12	13.67	1.37E+07	
		1.E-07	1	4	1	2.00	2.00E+07	
66		1.E-04	TNTC	TNTC	TNTC			
		1.E-05	153	165	150	156.00	1.56E+07	-0.007
		1.E-06	14	25	20	19.67	1.97E+07	
67	10	1.E-04	TNTC	TNTC	TNTC			
		1.E-05	106	102	105	104.33	1.04E+07	0.168
		1.E-06	9	15	7	10.33	1.03E+07	
68		1.E-04	TNTC	TNTC	TNTC			
		1.E-05	116	99	110	108.33	1.08E+07	0.152
		1.E-06	12	14	16	14.00	1.40E+07	
69		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	TNTC	TNTC	TNTC			
		1.E-05	116	110	110	112.00	1.12E+07	0.137
70	20	1.E-03	TNTC	TNTC	TNTC			
		1.E-04	TNTC	TNTC	TNTC			
		1.E-05		38	32	35.00	3.50E+06	0.642
71		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	TNTC	TNTC	TNTC			
		1.E-05	26	29	37	30.67	3.07E+06	0.700
72		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	315	335	337	329.00	3.29E+06	0.669
73	40	1.E-02	TNTC	TNTC	TNTC			
		1.E-03	83	95	86	88.00	8.80E+04	2.242
		1.E-04		6	7	6.50	6.50E+04	
74		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	172	151	170	164.33	1.64E+05	1.971
		1.E-04	12	19	15	15.33	1.53E+05	
75		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	119	105	124	116.00	1.16E+05	2.122
		1.E-04	7	12	12	10.33	1.03E+05	

Table C- 6 Data for UV inactivation of *B. subtilis* spores seeded into pH 8 phosphate buffered DI water. Spores were homogenized before seeding into raw water. These data were plotted in Figures 4-10, 4-12 and 4-14.

Run No.	Dose (mJ/cm^2)	Dilution	Count 1	Count 2	Count 3	Average Count	CFU/mL	log Inactivation
76	0	1.E-04	TNTC	TNTC	TNTC			
		1.E-05	162	169	163	164.67	1.65E+07	
		1.E-06	24	14	16	18.00	1.80E+07	
77		1.E-05	126	121	119	122.00	1.22E+07	1.52E+07
		1.E-06	12	11	13	12.00	1.20E+07	
		1.E-07	3	0	0	1.00	1.00E+07	
78		1.E-05	174	165	172	170.33	1.70E+07	
		1.E-06	15	21	15	17.00	1.70E+07	
		1.E-07	1	2	6	3.00		
79	5	1.E-05	145	132	140	139.00	1.39E+07	0.040
		1.E-06	9	16	13	12.67	1.27E+07	
		1.E-07	2	0	1	1.00	1.00E+07	
80		1.E-05	174	176	179	176.33	1.76E+07	-0.064
		1.E-06	18	19	15	17.33	1.73E+07	
		1.E-07	3	1	0	1.33	1.33E+07	
81		1.E-05	92	78	88	86.00	8.60E+06	0.248
		1.E-06	10	9	3	7.33	7.33E+06	
		1.E-07	1	0	2	1.00	1.00E+07	
82	10	1.E-04	TNTC	TNTC	TNTC			
		1.E-05	105	107	103	105.00	1.05E+07	0.162
		1.E-06	9	8	11	9.33	9.33E+06	
83		1.E-04	TNTC	TNTC	TNTC			
		1.E-05	129	137	139	135.00	1.35E+07	0.052
		1.E-06	14	13	18	15.00	1.50E+07	
84		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	TNTC	TNTC	TNTC			
		1.E-05	121	125	125	123.67	1.24E+07	0.091
85	20	1.E-03	TNTC	TNTC	TNTC			
		1.E-04	TNTC	TNTC	TNTC			
		1.E-05	27	32	34	31.00	3.10E+06	0.691
86		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	TNTC	TNTC	TNTC			
		1.E-05	38	53	43	44.67	4.47E+06	0.533
87		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	265	295	255	271.67	2.72E+06	0.749
88	40	1.E-01	TNTC	TNTC	TNTC			
		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	51	52	38	47.00	4.70E+04	2.511
89		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	69	66	77	70.67	7.07E+04	2.334
		1.E-04	4	4	7	5.00	5.00E+04	
90		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	82	63	58	67.67	6.77E+04	2.352

Table C- 7 Data for UV inactivation of *B. subtilis* spores in filtered water that was *not homogenized*. These data were plotted in Figure 4-13.

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
91	0	1.E-01	TNTC	TNTC	TNTC			
		1.E-02	91	78	90	86.33	8.63E+03	
		1.E-03	7	4	5	5.33	5.33E+03	
92		1.E-01	TNTC	TNTC	TNTC			1.16E+04
		1.E-02	119	135	138	130.67	1.31E+04	
		1.E-03	13	8	9	10.00	1.00E+04	
93		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	130	140	126	132.00	1.32E+04	
		1.E-03	11	13	19	14.33	1.43E+04	
94	5	1.E-01	TNTC	TNTC	TNTC			
		1.E-02	85	101	115	100.33	1.00E+04	0.064
		1.E-03	10	10	7	9.00	9.00E+03	
95		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	134	138	121	131.00	1.31E+04	-0.052
		1.E-03	8	17	14	13.00	1.30E+04	
96		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	68	69	64	67.00	6.70E+03	0.240
		1.E-03	4	11	5	6.67	6.67E+03	
97	10	1.E+00	TNTC	TNTC	TNTC			
		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	106	110	106	107.33	1.07E+04	0.035
98		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	105	116	101	107.33	1.07E+04	0.035
99		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	99	113	109	107.00	1.07E+04	0.036
100	20	1.E+00	TNTC	TNTC	TNTC			
		1.E-01	256	226	263	248.33	2.48E+03	0.671
		1.E-02	25	28	30	27.67	2.77E+03	
101		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	250	299	286	278.33	2.78E+03	0.621
		1.E-02	21	26	23	23.33	2.33E+03	
102		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	31	28	35	31.33	3.13E+03	0.570
103	40	1.E+00	71	84	92	82.33	8.23E+01	2.150
		1.E-01	11	8	13	10.67	1.07E+02	
		1.E-02	0	0	0	0.00	0.00E+00	
104		1.E+00	89	96	90	91.67	9.17E+01	2.103
		1.E-01	5	12	9	8.67	8.67E+01	
		1.E-02	1	1	1	1.00	1.00E+02	
105		1.E+00	73	67	84	74.67	7.47E+01	2.193
		1.E-01		9	3	6.00	6.00E+01	
		1.E-02	0	0	1	0.33	3.33E+01	

Table C- 8 Data for UV inactivation of *B. subtilis* spores in filtered drinking water that was *homogenized after* UV exposure. These data were plotted in Figure 4-12, 4-13, and 4-14.

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
106	0	1.E-01	TNTC	TNTC	TNTC			
		1.E-02	32	30	35	32.33	3.23E+03	
		1.E-03	7	4	5	5.33	5.33E+03	
107		1.E-01	TNTC	TNTC	TNTC			2.88E+03
		1.E-02	44	31	19	31.33	3.13E+03	
		1.E-03	4	5	1	3.33	3.33E+03	
108		1.E-01	239	213	233	228.33	2.28E+03	
		1.E-02	33	21	19	24.33	2.43E+03	
		1.E-03	5	5	2	4.00		
109	5 + H	1.E-01	236	208	213	219.00	2.19E+03	0.119
		1.E-02	24	25	23	24.00	2.40E+03	
		1.E-03	3	1	1	1.67	1.67E+03	
110		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	37	46	34	39.00	3.90E+03	-0.131
		1.E-03	2	8	4	4.67	4.67E+03	
111		1.E-01	268	289	256	271.00	2.71E+03	0.027
		1.E-02	27	25	33	28.33	2.83E+03	
		1.E-03	1	3	3	2.33	2.33E+03	
112	10 + H	1.E+00	TNTC	TNTC	TNTC			
		1.E-01	179		186	182.50	1.83E+03	0.199
		1.E-02	24	23	17	21.33	2.13E+03	
113		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	213	221	254	229.33	2.29E+03	0.099
		1.E-02	17	31	16	21.33	2.13E+03	
114		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	TNTC	TNTC	TNTC			
		1.E-02	40	40	55	45.00	4.50E+03	-0.193
115	20 + H	1.E+00	TNTC	TNTC	TNTC			
		1.E-01	56	46	45	49.00	4.90E+02	0.770
		1.E-02	5	6	6	5.67	5.67E+02	
116		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	96	91	103	96.67	9.67E+02	0.475
		1.E-02	22	27	28	25.67	2.57E+03	
117		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	167	139	163	156.33	1.56E+03	0.266
		1.E-02	16	20	13	16.33	1.63E+03	
118	40 + H	1.E+00	28	30	31	29.67	2.97E+01	0.988
		1.E-01	4	4	3	3.67	3.67E+01	
		1.E-02	0	1	1	0.67	6.67E+01	
119		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	42	40	33	38.33	3.83E+02	0.876
		1.E-02	3	3	5	3.67	3.67E+02	
120		1.E+00	TNTC	TNTC	TNTC			
		1.E-01	96	110	118	108.00	1.08E+03	0.426
		1.E-02	8	14	7	9.67	9.67E+02	

Table C- 9 Data for UV inactivation of *B. subtilis* spores in backwash water that *homogenized after* UV exposure. These data were plotted in Figures 4-10 and 4-11.

Run No.	Dose (mJ/cm^2)	Dilution	Count			Average Count	CFU/mL	log Inactivation
			1	2	3			
121	0	1.E-04	46	64	47	52.33	5.23E+05	
		1.E-05	4	8	2	4.67	4.67E+05	
		1.E-06	0	1	1	0.67	6.67E+05	
122		1.E-04	95	94	97	95.33	9.53E+05	6.73E+05
		1.E-05	6	12	5	7.67	7.67E+05	
		1.E-06	0	2	1	1.00	1.00E+06	
123		1.E-04	54	55	54	54.33	5.43E+05	
		1.E-05	5	6	5	5.33	5.33E+05	
		1.E-06	0	0	0	0.00	0.00E+00	
124	5	1.E-04	44	31	28	34.33	3.43E+05	0.293
		1.E-05	2	3	2	2.33	2.33E+05	
		1.E-06	0	0	0	0.00	0.00E+00	
125		1.E-04	35	40	40	38.33	3.83E+05	0.245
		1.E-05	1	5	3	3.00	3.00E+05	
		1.E-06	1	1	0	0.67	6.67E+05	
126		1.E-04	27	21	19	22.33	2.23E+05	0.479
		1.E-05	1	2	1	1.33	1.33E+05	
		1.E-06	44	43	49	45.33	4.53E+07	
127	10	1.E-03	TNTC	TNTC	TNTC			
		1.E-04	61	54	34	49.67	4.97E+05	0.132
		1.E-05	5	8	2	5.00	5.00E+05	
128		1.E-03	236	207	214	219.00	2.19E+05	0.488
		1.E-04	20	24	20	21.33	2.13E+05	
		1.E-05	5	3	2	3.33	3.33E+05	
129		1.E-03	311	284	313	302.67	3.03E+05	0.347
		1.E-04	20	26	23	23.00	2.30E+05	
		1.E-05	1	6	1	2.67	2.67E+05	
130	20	1.E-03	TNTC	TNTC	TNTC			
		1.E-04	46	35	38	39.67	3.97E+05	0.230
		1.E-05	1	3	2	2.00	2.00E+05	
131		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	63	69	67	66.33	6.63E+05	0.006
		1.E-05	11	11	4	8.67	8.67E+05	
132		1.E-03	281	282	264	275.67	2.76E+05	0.388
		1.E-04	20	23	24	22.33	2.23E+05	
		1.E-05	5	1	2	2.67	2.67E+05	
133	40	1.E-02	TNTC	TNTC	TNTC			
		1.E-03	136	157	172	155.00	1.55E+05	0.638
		1.E-04	16	9	22	15.67	1.57E+05	
134		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	TNTC	TNTC	TNTC			
		1.E-04	26	36	34	32.00	3.20E+05	0.323
135		1.E-02	TNTC	TNTC	TNTC			
		1.E-03	229	264	212	235.00	2.35E+05	0.457
		1.E-04	26	30	26	27.33	2.73E+05	

APPENDIX D EXPERIMENTAL DATA AND INFORMATION FROM MEMBRANE FILTRATION.

Table D- 1 Particle counts obtained from the filtrate of a 10 μm polycarbonate track etched filter (PCTE).

Nominal Size, μm	Particle Counts					
	Replicate 1		Replicate 2		Replicate 3	
	Cumulative	Differential	Cumulative	Differential	Cumulative	Differential
2	693.03	613.7	723.7	650	666	564.9
5	79.333	57.467	73.5	42.567	101.1	55.1
7	21.867	12.567	30.933	14.9	46	17.5
10	9.3	4.133	16.033	8.2	28.5	11.133
14	5.167	2.5	7.833	4.267	17.367	8.767
20	2.667	2.167	3.567	3.133	8.6	7.167
40	0.5	0.5	0.433	0.367	1.433	1.433
80	0	0	0.067	0.067	0	0

Table D- 2 Particle counts obtained from the filtrate of a 5 μm polycarbonate track etched filter (PCTE).

Nominal Size, μm	Particle Counts					
	Replicate 4		Replicate 5		Replicate 6	
	Cumulative	Differential	Cumulative	Differential	Cumulative	Differential
2	1649.7	1456.6	315.63	294.5	2481.9	1702.4
5	193.07	142.57	21.133	10.5	779.5	397.9
7	50.5	34.4	10.633	3.067	381.6	195.13
10	16.1	9.033	7.567	2.967	186.47	118.3
14	7.067	3.567	4.6	2.267	68.167	50.5
20	3.5	3.033	2.333	1.967	17.667	16.1
40	0.467	0.467	0.367	0.367	1.567	1.567
80	0	0	0	0	0	0

Nominal Size, μm	Replicate 19	
	Cumulative	Differential
2	834.73	770.77
5	63.967	41.133
7	22.833	11.8
10	11.033	5.7
14	5.333	3.5
20	1.833	1.4
40	0.433	0.433
80	0	0

Table D- 3 Particle counts obtained from the filtrate of a 2 µm polycarbonate track etched filter (PCTE) in triplicates.

Nominal Size, µm	Particle Counts					
	Replicate 7		Replicate 8		Replicate 9	
	Cumulative	Differential	Cumulative	Differential	Cumulative	Differential
2	70.733	48.567	434.57	256.9	165.6	94.8
5	22.167	9.733	177.67	73.733	70.8	44.767
7	12.433	4.6	103.93	43.6	26.033	15.833
10	7.833	3.9	60.333	32.833	10.2	3.833
14	3.933	1.867	27.5	17.633	6.367	3.633
20	2.067	1.7	9.867	8.667	2.733	2.4
40	0.367	0.333	1.2	1.2	0.333	0.333
80	0.033	0.033	0	0	0	0

Table D- 4 Particle counts obtained from the filtrate of a 0.4 µm polycarbonate track etched filter (PCTE) in triplicates.

Nominal Size, µm	Particle Counts					
	Replicate 10		Replicate 11		Replicate 12	
	Cumulative	Differential	Cumulative	Differential	Cumulative	Differential
2	71.5	48.3	116.13	93.533	110.03	82.267
5	23.2	9.933	22.6	13.833	27.767	15.2
7	13.267	4.5	8.767	3.967	12.567	5.5
10	8.767	4.267	4.8	2.2	7.067	3.467
14	4.5	2	2.6	1.2	3.6	2.067
20	2.5	2	1.4	1.133	1.533	1.233
40	0.5	0.5	0.267	0.267	0.3	0.3
80	0	0	0	0	0	0

Table D- 5 Particle counts obtained for sand media filter effluent.

Nominal Size, µm	Particle Counts					
	Replicate 13		Replicate 14		Replicate 15	
	Cumulative	Differential	Cumulative	Differential	Cumulative	Differential
2	307.63	267.5	371.83	317.3	1008.4	861.73
5	40.133	26	54.533	31.867	146.7	91.633
7	14.133	6.9	22.667	10.667	55.067	32.6
10	7.233	3.067	12	4.667	22.467	14.533
14	4.167	2.467	7.333	4.3	7.933	5.433
20	1.7	1.267	3.033	2.533	2.5	2.233
40	0.433	0.433	0.5	0.5	0.267	0.233
80	0	0	0	0	0.033	0.033

Nominal Size, µm	Replicate 18	
	Cumulative	Differential
2	1675.5	1428.9
5	246.57	154.43
7	92.133	52.533
10	39.6	25.733
14	13.867	10.2
20	3.667	3.4
40	0.267	0.267
80	0	0

Table D- 6 Particle counts obtained for Phosphate buffered DI water used to flush detector before particle counts were taken.

Nominal Size, µm	Particle Counts			
	Replicate 16		Replicate 17	
	Cumulative	Differential	Cumulative	Differential
2	44.1	30.933	40	17.033
5	13.167	5	22.967	5.9
7	8.167	2.767	17.067	5.167
10	5.4	1.667	11.9	3.9
14	3.733	1.7	8	3.4
20	2.033	1.7	4.6	3.633
40	0.333	0.333	0.967	0.967
80	0	0	0	0

Table D- 7 Concentration of *B. subtilis* spores that were washed off PCTE filters. These data are presented in Table 4-6.

Sample	Run No.	Dilution	Count			Average Count	CFU/mL
			1	2	3		
≥ 10 µm	1	1E+00	134	162	139	145.00	1.45E+02
		1E-01	11	12	14	12.33	1.23E+02
		1E-02	6	2	0	2.67	2.67E+02
	2	1E+00	145	154	160	153.00	1.53E+02
		1E-01	6	12	5	7.67	7.67E+01
		1E-02	0	0	0	0.00	0.00E+00
	3	1E+00	TNTC	TNTC	TNTC		
		1E-01	44	36	41	40.33	4.03E+02
		1E-02	3	3	4	3.33	3.33E+02
≥ 5 ≤ 10 µm	4	1E+00	TNTC	TNTC	TNTC		
		1E-01	22	25	34	27.00	2.70E+02
		1E-02	5	3	0	2.67	2.67E+02
	5	1E+00	TNTC	TNTC	TNTC		
		1E-01	44	48	43	45.00	4.50E+02
		1E-02	2	2	2	2.00	2.00E+02
	6	1E+00	181	194	189	188.00	1.88E+02
		1E-01	15	19	13	15.67	1.57E+02
		1E-02	0	2	0	0.67	6.67E+01
	18	1E+00	TNTC	TNTC	TNTC		
		1E-01	34	42	33	36.33	3.63E+02
		1E-02	2	3	3	2.67	2.67E+02
≥ 2 ≤ 5 µm	7	1E-01	47	49	48	48.00	4.80E+02
		1E-02	6	8	10	8.00	8.00E+02
		1E-03	0	0	0	0.00	0.00E+00
	8	1E-01	97	102	82	93.67	9.37E+02
		1E-02	7	8	4	6.33	6.33E+02
		1E-03	0	1	2	1.00	1.00E+03
	9	1E-01	123	108	106	112.33	1.12E+03
		1E-02	11	6	8	8.33	8.33E+02
		1E-03					
≥ 0.4 ≤ 2 µm	10	1E-01	TNTC	TNTC	TNTC		
		1E-02	29	39	35	34.33	3.43E+03
		1E-03	3	4	5	4.00	4.00E+03
	11	1E-01	270	238	251	253.00	2.53E+03
		1E-02	15	12	16	14.33	1.43E+03
		1E-03	0	0	1	0.33	3.33E+02
	12	1E-01	TNTC	TNTC	TNTC		
		1E-02	21	31	36	29.33	2.93E+03
		1E-03	1	0	1	0.67	6.67E+02

Table D- 8 Concentration of *B. subtilis* spores in sand media effluents prior to filtration through PCTE filters. These data is presented in 4-15.

Sample	Run No.	Dilution	Count			Average Count	CFU/mL
			1	2	3		
Media Filtered Water	13 NH[a]	1E-01	TNTC	TNTC	TNTC		
		1E-02	149	149	152	150.00	1.50E+04
		1E-03	26	14	17	19.00	1.90E+04
	13H[b]	1E-01	TNTC	TNTC	TNTC		
		1E-02	154	124	124	134.00	1.34E+04
		1E-03	13	12	16	13.67	1.37E+04
	14NH	1E-01	TNTC	TNTC	TNTC		
		1E-02	155	159	161	158.33	1.58E+04
		1E-03	20	19	16	18.33	1.83E+04
	14H	1E-01	TNTC	TNTC	TNTC		
		1E-02	264	261	253	259.33	2.59E+04
		1E-03	35	22	24	27.00	2.70E+04
	15NH	1E-01	TNTC	TNTC	TNTC		
		1E-02	162	173	159	164.67	1.65E+04
		1E-03	20	26	21	22.33	2.23E+04
	15H	1E-01	TNTC	TNTC	TNTC		
		1E-02	150	155	150	151.67	1.52E+04
		1E-03	23	14	12	16.33	1.63E+04
Controls	16	1E+00	0	0	0	0.00	0.00E+00
	17	1E+00	0	0	0	0.00	0.00E+00

[a] NH – Samples were not homogenized before enumeration

[b] H – Samples were homogenized before enumeration.

Number Distributions (Lawler et al. 1980)

$$\frac{dN}{d(\log d_p)} = 2.3 d_p n(d_p) \qquad \text{Equation E-1}$$

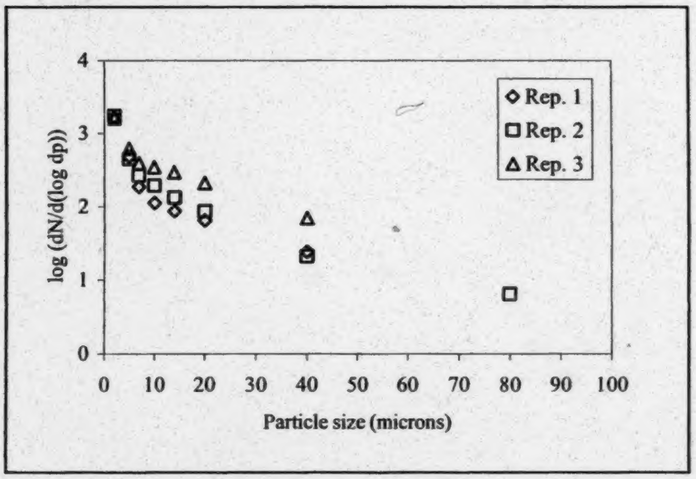

Figure D- 1 Number distribution of particles that passed through a 10 µm PCTE membrane filter.

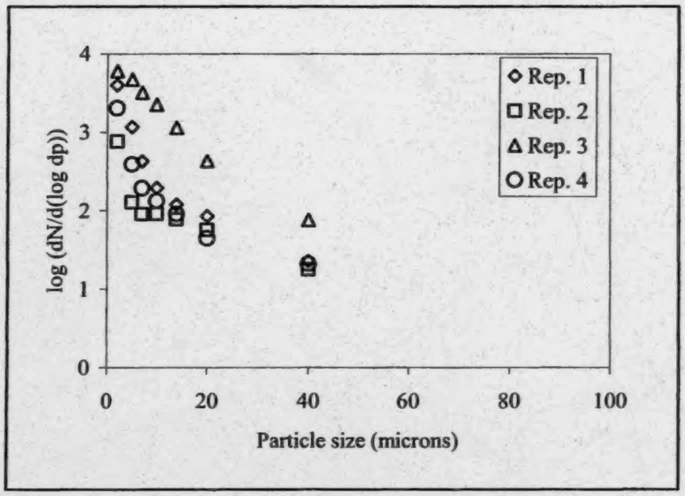

Figure D- 2 Number distribution of particles that passed through a 5 µm PCTE membrane filter.

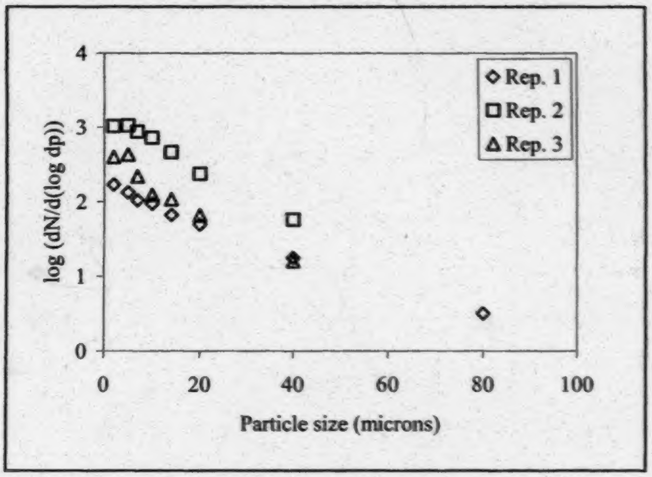

Figure D- 3 Number distribution of particles that passed through a 2 µm PCTE membrane filter.

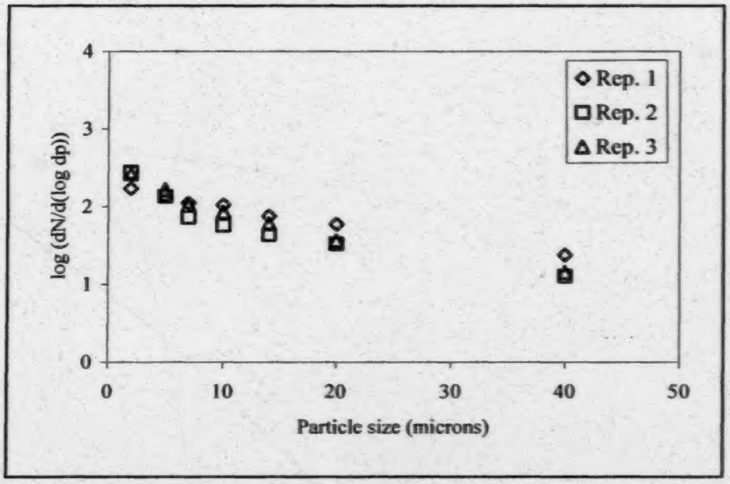

Figure D- 4 Number distribution of particles that passed through a 0.4 µm PCTE membrane filter.

APPENDIX E SCANNING ELECTRON MICROGRAPHS

E-1 MICROGRAPHS FROM SCANNING ELECTRON MICROSCOPE

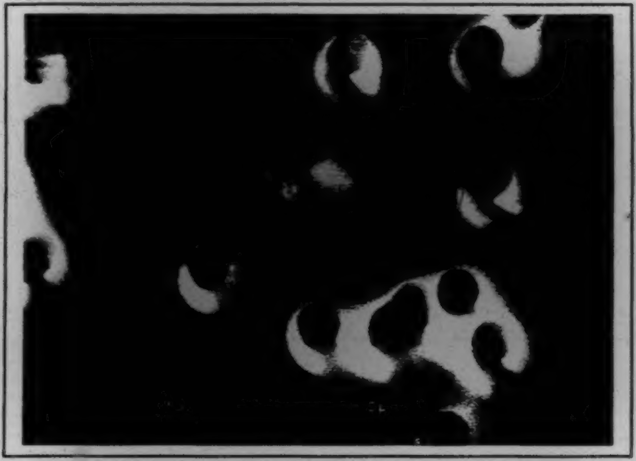

Figure E- 1 SEM micrographs showing aggregated *B. subtilis* spores on a 2 μm PCTE membrane filter.

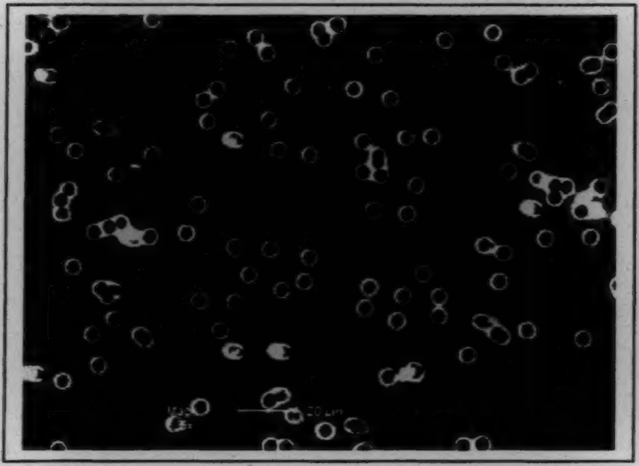

Figure E- 2 SEM micrographs showing a 5 μm PCTE filter.

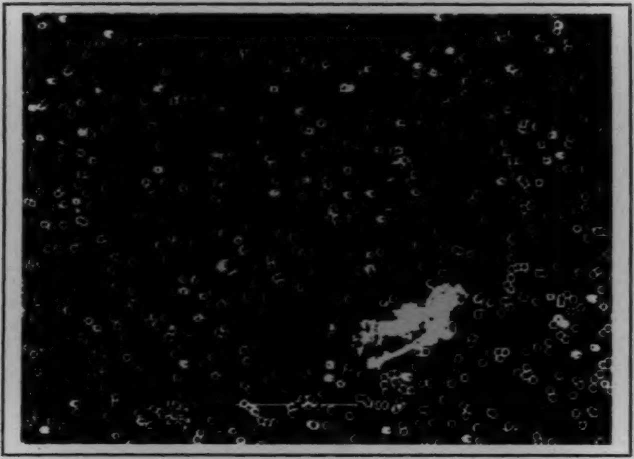

Figure E- 3 SEM micrograph showing a 5 μm PCTE membrane filter. Large floc material 50 to 80 μm in size was captured on filter.

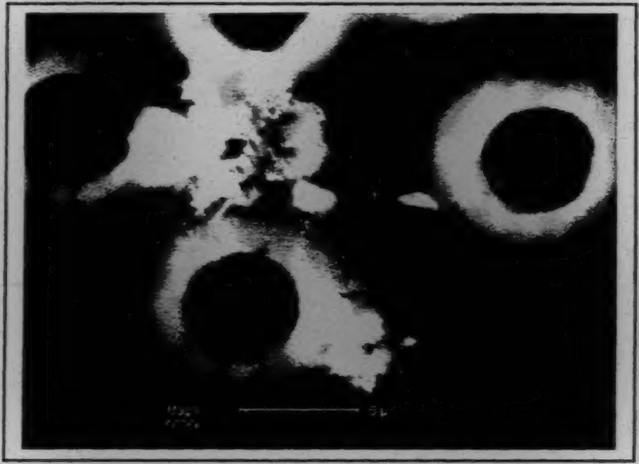

Figure E- 4 SEM micrograph showing a 5 μm PCTE membrane filter. Floc holding spore and other material together.

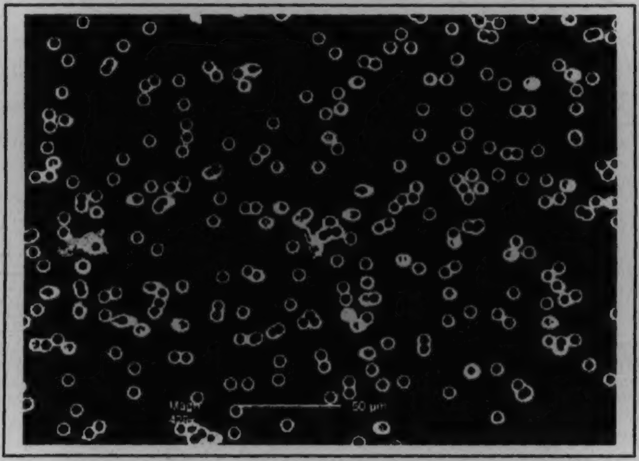

Figure E- 5 SEM micrograph showing a 5 µm PCTE membrane filter.